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Human Microsporidial Infections

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INTRODUCTION

The term microsporidia refers to a group of obligate intracellular protozoal parasites belonging to the phylum *Microspora*. Since 1857, when Nägeli first discussed newly identified parasitic agents in silkworms, which he termed *Nosema bombycis* (140), microsporidia have been recognized as a cause of disease in various animal groups, among them commercially important insects, fish, laboratory rodents, rabbits, fur-bearing animals, and primates (45, 82, 141, 171, 194, 252). Indeed, their host range is extensive, including most invertebrates and all classes of vertebrates (45, 200–203). More than 100 microsporidial genera and almost 1,000 species have now been identified (37, 201). Microsporidia have also been found in common environmental sources such as ditch water (6). Furthermore, they are being investigated as biological control agents against certain invertebrate vectors of disease and pests such as grasshoppers and locusts (91).

The first human case of sufficiently substantiated microsporidial infection was reported in 1959 (127). Subsequently, only nine additional cases have been described among persons not infected with the human immunodeficiency virus (HIV) (Table 1) (25, 26, 42, 175, 176). Yet, as part of the evolving pandemic of HIV infection, microsporidia have gained attention as opportunistic pathogens (29, 42, 191, 192). Increased awareness of these organisms is related to the development of improved diagnostic methods. Three new microsporidial species, *Enterocytozoon bieneusi* in 1985 (66), *Encephalitozoon hellem* in 1991 (68), and *Septata intestinalis* in 1993 (31, 34), have been identified in patients with AIDS (Table 2), and over 400 cases of HIV-associated microsporidial infection have been documented (25, 29). To date, five genera (*Enterocytozoon* spp., *Encephalitozoon* spp., *Septata* spp., *Pleistophora* sp., and *Nosema* spp.), as well as unclassified microsporidial organisms (referred to by the collective term *Microsporidium*), have been associated with human disease, which appears to predominantly affect immunocompromised persons (25, 29, 37, 42, 44, 45). The potential sources and means of transmission of human microsporidial infections are uncertain.

Preliminary communications have indicated that microsporidial species first identified in patients with AIDS will not be confined to this patient group. *Enterocytozoon bieneusi* has recently been detected as a cause of self-limiting diarrhea in an immunocompetent traveler (175), as well as in a patient who was immunosuppressed secondary to an organ transplantation (176).

THE ORGANISMS

Biologic Characteristics

Microsporidia are small unicellular parasites that are considered true eukaryotes because they have a nucleus with a nuclear envelope, an intracytoplasmic membrane system, and chromosome separation on mitotic spindles (45). Microsporidia also share prokaryotic features, indicating that they are phylogenetically very ancient protozoa (58, 227). Specifically, microsporidia lack eukaryotic ribosomal characteristics; the small rRNA is of prokaryotic size; and they lack mitochondria, peroxisomes, and Golgi membranes (227).

Microsporidia develop intracellularly exclusively and have no metabolically active stages outside the host cell (37). A unique life cycle involving a proliferative merogonic stage followed by a sporogonic stage results in distinctive and resistant infective spores. Mature spores contain an exceptional tubular extrusion apparatus for injecting infective spore contents into the host cell. These spores are the most characteristic stages by which microsporidia are identified and distinguished from other organisms (30, 35, 37).

The development of some microsporidial species is confined to a specific host cell of a single organ system; other species cause systemic infection involving different organ systems. Manifestations of microsporidial infections may depend on the infecting species, mode of infection, age of the host at the time of infection, and the competence of the host's immune response. Imbalance of the parasite-host interaction results in

TABLE 1. Synopsis of case reports of microsporidiosis in patients without HIV infection

Microsporidial species	Patient statistics[a]			Clinical manifestation	Immune status	Detection of parasite	Reference	
	Age (yr)	Sex	Location				No.	Yr
Encephalitozoon cuniculi	9	M	Japan	Seizures	Not known; anergy to tuberculin after BCG	Cerebrospinal fluid, urine	127	1959
Encephalitozoon cuniculi	2	M	Sweden[b]	Seizures	Low $CD4^+/CD8^+$ cell ratio	Serum antibody, urine	11	1984
Nosema connori	4 mo	M	USA	Disseminated infection	Thymic aplasia	Autopsy	125	1973
Microsporidium ceylonensis	11	M	Sri Lanka	Corneal ulcer	Not known	Histological examination	5	1973
Microsporidium africanum	26	F	Botswana	Corneal ulcer	Not known	Histological examination	161	1981
Pleistophora sp.	20	M	USA	Myositis	Cellular immune-deficiency (HIV antibody negative)	Histological examination	116, 123	1985
Nosema corneum	45	M	USA	Keratitis	Normal	Histological examination	61, 193	1990
Nosema ocularum	39	M	USA	Keratitis	Normal	Histological examination	26, 32	1991
Enterocytozoon bieneusi	26	M	Germany[c]	Self-limited diarrhea	Normal	Stool specimen	175	1994
Enterocytozoon bieneusi		F	USA	Self-limited diarrhea	Immunosuppression after liver transplantation	Stool specimen	176	1993

[a] F, female; M, male. USA, United States of America.
[b] The boy was born in Colombia.
[c] The patient had travelled in Egypt and Jordan.

proliferation and dissemination of the parasites, causing destruction of host cells.

Taxonomy

The term microsporidia is a nontaxonomic designation commonly used to describe the group of organisms belonging to the phylum *Microspora*, which is contained within the subkingdom Protozoa (28, 45, 200). Since 1882, when Balbiani classified the novel parasitic organisms as a separate group which he named "Microsporidies" (7, 111), the taxonomy of microsporidia has been subjected to several modifications, and it will probably be amended significantly when results of comprehensive molecular analyses become available. Until recently, Sprague's classification system, proposed in 1977 and updated in 1982, was the most widely used (120, 200, 203). Taxonomy and species classification have primarily been based on ultrastructural features, i.e., the size and morphology of the different developmental stages, the configuration of the nuclei in spores and developmental stages, the host-parasite interface, and the number of coils of the tubular extrusion apparatus of the spores. Species characteristics also included host cell

TABLE 2. Microsporidial species and associated clinical manifestations in HIV-infected patients

Species	Clinical manifestation(s)	First report(s)	
		Reference(s)	Yr
Enterocytozoon bieneusi	Diarrhea, wasting syndrome	66, 74, 135	1985
	Cholecystitis, cholangitis	131, 163	1991
	Bronchitis, pneumonia[a]	234	1992
	Sinusitis, rhinitis[a]	76, 90	1992
Encephalitozoon cuniculi	Fulminant hepatitis[a,b]	207	1987
	Peritonitis[a,b]	253	1989
	Disseminated infection[a]	62	1994
Encephalitozoon, species not designated[b]	Keratoconjunctivitis	48	1990
	Sinusitis, nasal polyps	38, 113	1992
Encephalitozoon hellem	Keratoconjunctivitis, conjunctivitis	68	1991
	Disseminated infection (tubulointerstitial nephritis, ureteritis, cystitis, keratoconjunctivitis, colonization of bronchial epithelium)	180	1991
	Bronchiolitis, pneumonia[a]	184	1993
Septata intestinalis	Diarrhea	34	1991
	Disseminated infection (tubulointerstitial nephritis, diarrhea, cholecystitis)	31, 150, 153	1992, 1993
Pleistophora sp.	Myositis[a]	51	1993

[a] Only one case reported.
[b] Classification was based on morphology. Because morphological ultrastructure is similar in different species of *Encephalitozoon*, it is not known whether the classification was correct. It is also not known whether the different *Encephalitozoon* species reported in humans may be microsporidia of the same species.

TABLE 3. Taxonomy of microsporidia infecting humans (using Sprague's revised taxonomy, 1992 [201])[a]

Class	Order	Family	Genus	Species
Dihaplophasea	Meiodihaplophasida	(None infecting humans)		
	Dissociodihaplophasida	Nosematidae	*Nosema*	*Nosema connori*
				Nosema corneum
				Nosema ocularum
Haplophasea	Glugeida	Pleistophoridae	*Pleistophora*	*Pleistophora* sp.
		Encephalitozoonidae	*Encephalitozoon*	*Encephalitozoon hellem*
				Encephalitozoon cuniculi
				Encephalitozoon sp.
			Septata	*Septata intestinalis*
	Chytridiopsida	Enterocytozoonidae	*Enterocytozoon*	*Enterocytozoon bieneusi*
Not classified	Not classified	Not classified	Not classified	*Microsporidium ceylonensis*
				Microsporidium africanum

[a] Subkingdom: Protozoa; Phylum: *Microspora.*

specificity and patterns of organ, tissue, and cell involvement by the parasite. In 1992, Sprague and colleagues proposed a comprehensive revision in which differences in chromosome cycles were treated as the most fundamental taxonomic characteristics (Table 3). They deliberately avoided the subject of evolutionary relationships, because present knowledge is limited to a few molecular studies (201).

Ultrastructural examination of microsporidia may not be appropriate to indicate relationships of genera or to differentiate species within a genus. Didier and colleagues found consistent differences between *Encephalitozoon* spp. isolated from patients with AIDS and *Encephalitozoon cuniculi* of animal source when compared by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot (immunoblot) analyses, although the isolates were morphologically identical. These results led to the description of a new species, *Encephalitozoon hellem* (68). Most recently, molecular analyses of *Encephalitozoon hellem* and *Encephalitozoon cuniculi* isolates have shown differences in rDNA sequences that support the distinction of the two strains (226).

Taxonomy of microsporidia infecting humans is not conclusive at present. Some microsporidia that have been found in humans are not classified because the life cycle of the organism is not completely documented.

Life Cycle

Developmental stages. The life cycle of microsporidia includes three distinct phases: first, the infective phase, i.e., the spore stage, made up of spore release into the environment, spore transmission, stimulation of the spore necessary to trigger the extrusion of the polar tubule, and inoculation of infective spore content (termed sporoplasm) into a host cell; second, the proliferative vegetative phase, termed merogony (schizogony), during which the parasites multiply intracellularly; and third, the intracellular sporogony, during which infective spores are formed (30, 35, 45). Merogony and sporogony may take place simultaneously in the same multiply infected host cell. The structure of all developmental stages of microsporidia is cellular. The configuration of the nucleus differs among the genera. In some, the nucleus is single throughout the life cycle (e.g., *Encephalitozoon* spp.); in others, two nuclei are arranged as coupled pairs, termed diplokaryon (e.g., *Nosema* spp.). Also, both types of the nuclear configuration may occur at different phases of the life cycle of a genus (30, 35, 45, 112).

In human microsporidial infection, the life cycle of the organisms is completed within the human host, and there is no evidence of an intermediate host or a vector transmitting developmental stages of microsporidia (37). For some microsporidial genera infecting mosquitoes, complex developmental sequences that involve obligate alternation between different invertebrate hosts have been discovered (3, 206).

Infective spore. Whereas the developmental stages preceding the spore are structurally simple cells, the microsporidial spore is distinctively differentiated to transmit infectious material to a new host by a unique mechanism (Fig. 1 and 2). A thick wall consisting of an electron-dense proteinaceous exospore, an electron-lucent chitinous endospore layer, and a plasma membrane renders the spores environmentally resistant. Laboratory observations suggest that spores, e.g., those of *N. bombycis*, may remain viable for up to 10 years in distilled water (203). The spore wall encloses the uni- or binucleate infective spore content (sporoplasm), an exceptional extrusion

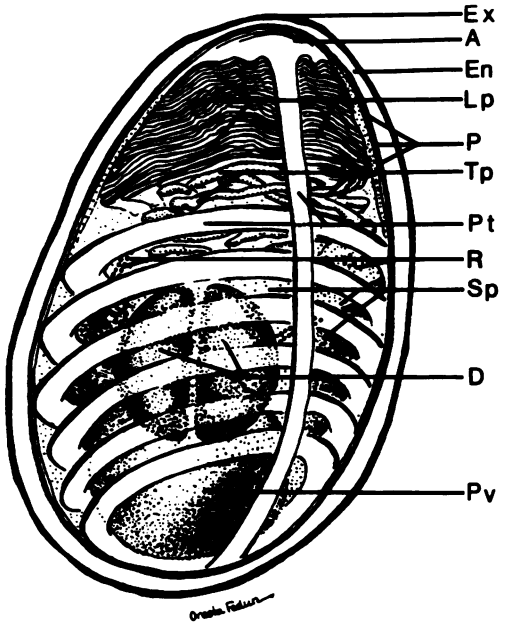


FIG. 1. Diagram of a microsporidial spore. The spore wall consists of an electron-dense exospore (Ex), an electron-lucent endospore (En), and a unit membrane (P). The extrusion apparatus includes an anchoring disc (A), polar tubule (Pt), lamellar polaroplast (Lp), and tubular polaroplast (Tp). Posterior vacuole (Pv), ribosomes (R), sporoplasm (Sp), and nucleus (D) are shown. From reference 35, with permission of the publisher.

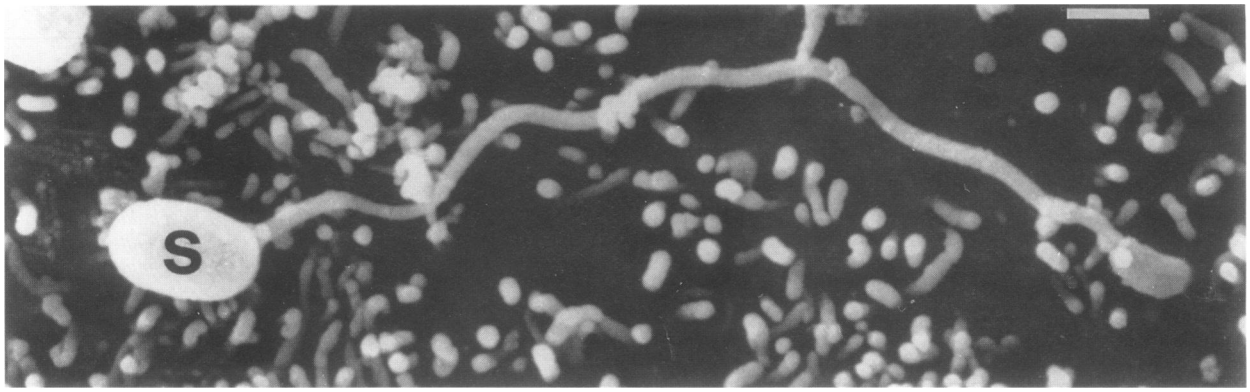


FIG. 2. Scanning electron micrograph of an *Encephalitozoon hellem* spore (S) with extruded polar tube with a paddle-shaped sporoplasm at the end of the tube. The microsporidian was obtained from urine of an HIV-infected patient with disseminated *E. hellem* infection and cultivated in vitro, using Vero green monkey kidney cell culture. Bar = 1 μm. From reference 223, with permission of the publisher.

apparatus (the polar tubule) for injecting the sporoplasm into new host cells, a complex membrane system (termed lamellar polaroplast) surrounding the straight section of the polar tubule, some rough endoplasmatic reticulum, and free ribosomes. The extrusion apparatus consists of a polar tubule that lies coiled inside the spore and is attached to an anchoring disk. The tubule is everted when triggered by appropriate environmental stimuli, e.g., small-intestinal fluid, and is capable of penetrating a host cell to inoculate the sporoplasm into the host cell cytoplasm (102, 162, 241). The location of the sporoplasm within the host cell is determined by the direction of penetration and length of the polar tubule.

Merogony. Sporoplasm injected into a host cell initiates the proliferative merogonic stage. Meronts are structurally simple cells with little differentiation of the cytoplasm and bounded by a unit-type membrane. They multiply repeatedly by binary and multiple fission. Karyokinesis (nuclear division) may occur repeatedly before cytokinesis (cell division), resulting in rounded multinucleate plasmodial forms (e.g., *Enterocytozoon bieneusi*) or in ribbonlike multinucleate cells (e.g., *S. intestinalis*) (31, 36).

Sporogony. Sporogony is initiated when meronts develop into sporonts that are morphologically characterized by the appearance of a dense amorphous surface coat around the cell. Sporonts grow and multiply by binary or multiple fission to form sporoblasts, which mature into spores by developing the distinct cytoplasmic organelles and continuous thickening of the wall.

Host-parasite interface. The contact between the host cell and microsporidia varies in genera infecting humans: all stages of *Enterocytozoon bieneusi* develop in direct contact with the host cell cytoplasm (36). Sporogony of both *Encephalitozoon* and *Septata* spp. occurs within a parasitophorous vacuole limited by a host-formed membrane (31, 37). *Pleistophora* sp. develops within a sporophorous vesicle which segregates the parasite from host cytoplasm by a thick parasite-formed membrane (45, 51, 116).

Cultivation

As microsporidia are obligate intracellular parasites, they cannot grow and multiply independently of their host cells. Many microsporidial species infecting animals can be maintained in the laboratory in their natural hosts (45). In vitro, microsporidia can be propagated in cell culture systems but not in an axenic medium (189, 193). Not all microsporidial species infecting humans, however, have been cultured (37, 42). The first microsporidian isolated from humans by Shadduck and colleagues, *Nosema corneum*, was obtained from the corneal stroma of a patient with keratitis who was HIV seronegative (193). The first *Encephalitozoon hellem* isolates were also obtained from ocular specimens, i.e., corneal tissue and conjunctival scrapings from patients with AIDS and keratoconjunctivitis (68). *Encephalitozoon hellem* was subsequently isolated from the urine (Fig. 3) (223) and bronchoalveolar lavage fluid (Fig. 4) (184) of HIV-infected patients with disseminated microsporidiosis. Preliminary data of cultivation of presumptive *S. intestinalis* from urine and feces of a patient with AIDS and diarrhea have also been reported (96). Different cell culture systems have been successfully used to isolate microsporidia (41). Although continuous propagation of *Enterocytozoon bieneusi* has not been attained, preliminary data indicate that maintenance of this parasite may be possible because short-term cell culture of up to 6 months has been accomplished (222).

Antigenic and Immunologic Characteristics

With SDS-PAGE separation techniques, proteins were extracted from spores of *Encephalitozoon* isolates obtained from conjunctival specimens and urine of patients with AIDS and *Encephalitozoon cuniculi* isolates of animal source. The protein bands ranged in molecular mass from 10 to 200 kDa (68, 223). Important and consistent differences were found between the protein patterns of human *Encephalitozoon* isolates and *Encephalitozoon cuniculi*, although parasite ultrastructure was identical (68, 223, 244). Also, Western blot immunodetection technique indicated that the patients' isolates represented one organism and were immunologically different from *Encephalitozoon cuniculi*, implying that isolates from patients with AIDS belonged to a separate species which was named *Encephalitozoon hellem* by Didier and colleagues (68).

Molecular Analysis

Small-subunit rRNA sequences are used for species-specific detection of organisms as well as for analysis of phylogenetic relationships of organisms. The first sequence data of small-subunit rRNA of an insect microsporidian, *Vairimorpha necatrix*, reported by Vossbrinck and colleagues indicated that evolutionary development leading to microsporidia branched very early from that leading to other eukaryotes (227). Nucleotide sequences of the small-subunit rRNA of eight microspo-

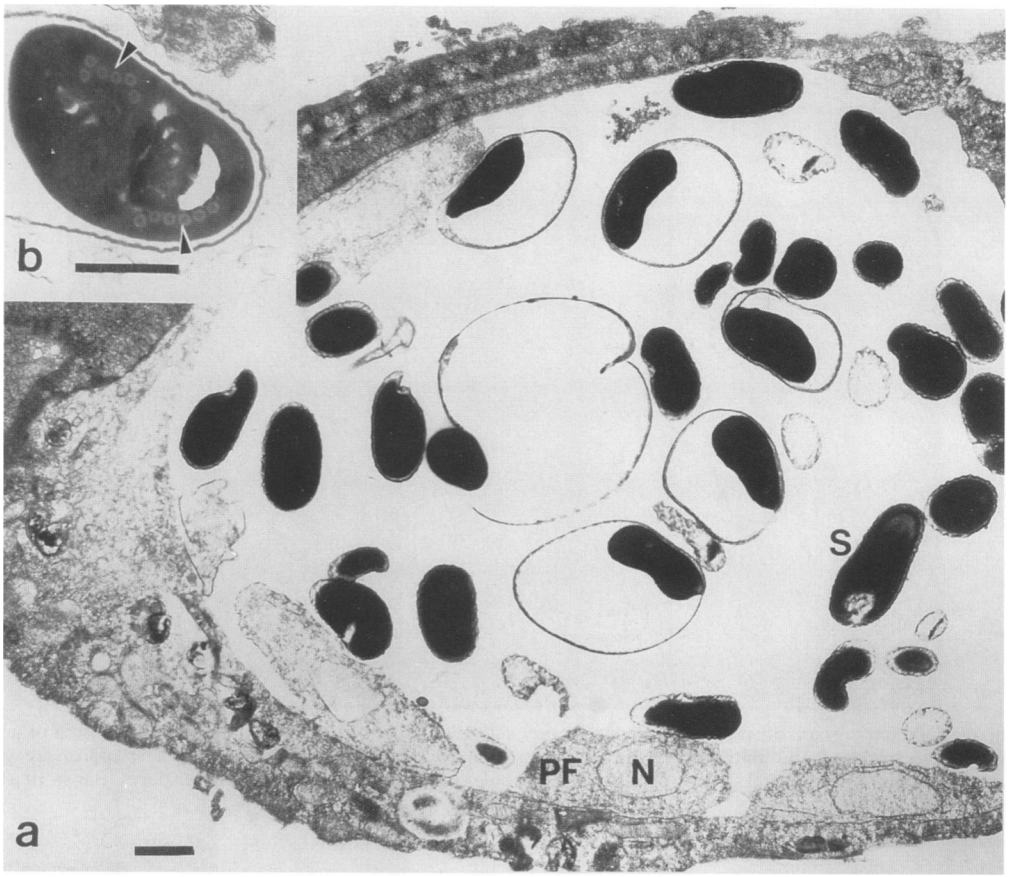


FIG. 3. Transmission electron micrograph of a human lung fibroblast cell depicting (a) proliferative forms (PF) of *Encephalitozoon hellem*, attached to the membrane of the parasitophorous vacuole, and mature spores (S) within the vacuole; (b) a mature spore with six cross sections of the polar tube (arrowheads). The microsporidian was obtained from urine of an HIV-infected patient with disseminated *E. hellem* infection. Bar = 1 μm. From reference 223, with permission of the publisher.

ridial species have been published or are accessible via the GenBank database (August 1993) (160) and should be helpful in developing diagnostic PCR primers which can be used as species-specific identification tools (256). Among them, sequence data of the species *Encephalitozoon cuniculi* (89, 160, 226, 254), *Encephalitozoon hellem* (160, 226), *Enterocytozoon bieneusi* (90, 256), *S. intestinalis* (255), and *Pleistophora* sp. (94) are available. Molecular analyses of *Encephalitozoon hellem* isolates from patients with AIDS and *Encephalitozoon cuniculi* from animal sources have shown a high sequence similarity of rDNA, supporting the classification of these two organisms in the same genus, but sequence differences also have confirmed that they are not the same species (226). Molecular analyses have also confirmed that *S. intestinalis* and *Encephalitozoon hellem* are distinct organisms (255).

GENUS- AND SPECIES-SPECIFIC MORPHOLOGY AND DEVELOPMENT

Enterocytozoon spp.

Enterocytozoon bieneusi, first described by Desportes and colleagues in 1985 (66), is primarily found in small-intestinal enterocytes of HIV-infected patients with chronic diarrhea (147). It has also been infrequently identified in other epithelial cells of patients with AIDS, i.e., in the biliary tree, gallbladder, nonparenchymal liver cells (163, 164), pancreatic duct (27), and tracheal (148), bronchial (234), and nasal (76, 90) epithelia. The only other *Enterocytozoon* species currently known, *Enterocytozoon salmonis*, is an intranuclear parasite of salmonid fish (50).

All developmental stages are formed in direct contact with the host cell cytoplasm, and no sporophorous vesicles or pansporoblastic membranes are present (Fig. 5 and 6). The parasite has no diplokarya in any stage of development and contains elongated nuclei during early development (unique to this genus). The proliferative and sporogonial forms are rounded multinucleate plasmodia measuring up to 6 μm in diameter and limited by a unit membrane. Other organelles unique to this genus are electron-lucent inclusions which are present throughout the life cycle and electron-dense discs which are formed during sporogony and represent precursors to the polar tube and anchoring discs. Sporoblasts develop from large plasmodial sporonts that are divided by invagination of the plasmalemma. The oval spores display typical microsporidial ultrastructure and measure 0.7 to 0.98 by 1.08 to 1.64 μm. The polar tubule has five to seven coils that appear in two rows when seen in cross sections by transmission electron microscopy (36).

Encephalitozoon spp.

Encephalitozoon cuniculi, first demonstrated in rabbits by Wright and Craighead in 1922 (250), and named by Levaditi, Nicolau, and Schoen in 1923 (119, 242), is parasitic in different

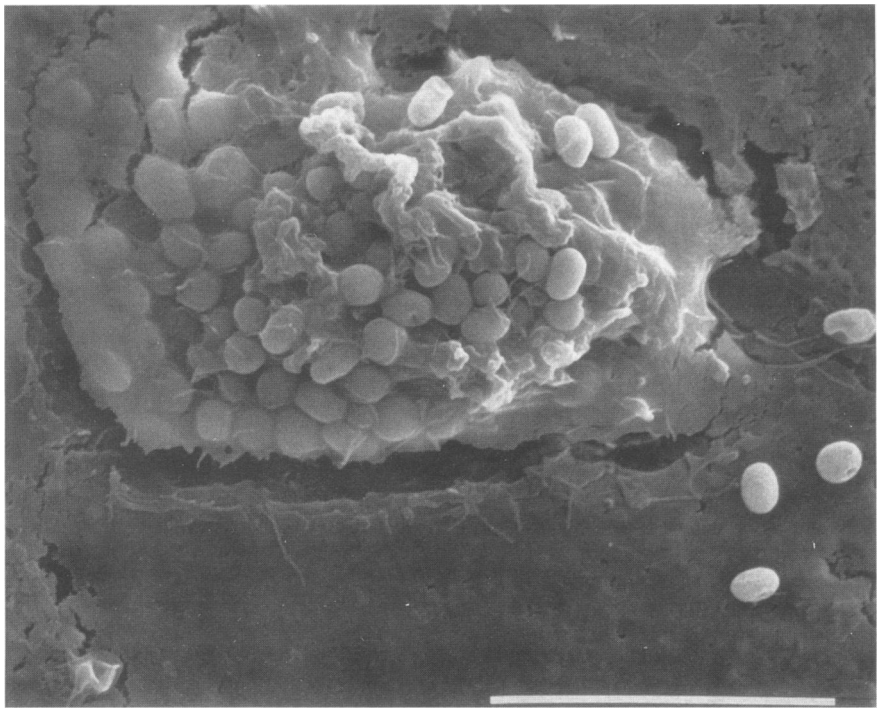


FIG. 4. Scanning electron micrograph of spores of *Encephalitozoon hellem* obtained from bronchoalveolar lavage fluid of a patient with AIDS and disseminated *E. hellem* infection and cultivated in vitro, using a monkey kidney cell culture system. The spores are concentrated in an intracytoplasmic parasitophorous vacuole, and slender extruded polar tubules are seen projecting from some spores. Bar = 10 μm. From reference 184, with permission of the publisher.

mammals, including rodents, carnivores, primates, and humans, as well as in birds (39, 42, 45, 141, 167). It infects macrophages, epithelial cells, vascular endothelial cells, kidney tubule cells, and possibly other cell types and can be found in most tissues, with a predilection for brain and kidney (45).

Some *Encephalitozoon* organisms found in patients with AIDS have recently been distinguished from *E. cuniculi* of animal source and were named *E. hellem* by Didier and colleagues in 1991 (68). *E. hellem* has been identified in macrophages, in corneal and conjunctival epithelial cells, nasal epithelium, bronchial epithelium, lining epithelium of the urinary tract, and renal tubules (68, 76, 180, 183, 184, 223). Whether previous reports of central nervous system infection due to *E. cuniculi* in two children not infected with HIV (11, 127), as well as liver (207) and peritoneal infection (253) in patients with AIDS, were indeed caused by this species, by another *Encephalitozoon*-like species, or possibly by *E. hellem* is not known because examination by protein and antigenic analyses or molecular studies were not performed in these cases.

Encephalitozoon spp. develop intracellularly in a phagosomelike parasitophorous vacuole bounded by a membrane of presumed host cell origin (Fig. 3) (156). Nuclei of all stages are unpaired. Meronts divide repeatedly by binary fission, lie close to the vacuolar membrane, and are rounded or oval and slightly irregular structures measuring approximately 2 to 6 by 1 to 3 μm. Sporonts appear free in the center of the vacuole and divide into two sporoblasts, which mature into spores. The spores of *E. hellem* measure 2.0 to 2.5 by 1.0 to 1.5 μm, and the polar tubule has five to seven coils, usually in single rows (68).

S. intestinalis

S. intestinalis, described and taxonomically classified by Cali and colleagues in 1993 (31, 34), has exclusively been identified in HIV-infected patients. It was first detected in enterocytes and lamina propria macrophages of patients with chronic diarrhea (34, 150, 153). In addition, parasites have also been detected in fibroblasts and endothelial cells of the lamina propria, as well as in epithelial cells of the biliary tree, tubular kidney cells, bronchial epithelial cells, and nasal epithelial cells (31, 79, 150, 153).

S. intestinalis has morphological and developmental characteristics comparable to those of *Encephalitozoon* spp. (31) (Fig. 7 to 9). Meronts of both genera proliferate by cellular elongation and cytoplasmic invagination between nuclei, and sporonts also divide by fission. Whereas only binucleate proliferative cells and binucleate sporonts are observed in *Encephalitozoon* sp., which is disporous, *S. intestinalis* is characterized by proliferative cells which are single-, bi-, or tetranucleated, and sporogony is tetrasporous. Sporogony of both *Encephalitozoon* and *Septata* spp. takes place intracellularly within a parasitophorous vacuole of host origin, but *Septata*-infected cells show a unique parasite-secreted fibrillar network surrounding the developing organisms so that the parasitophorous vacuole appears septate. Sections examined by electron microscopy may depict unique tubular appendages which are up to 1.2 μm in length and approximately 50 nm in diameter. They appear to originate from the sporont surface and terminate in an enlarged bulblike structure. The mature spores measure 1.2 by 2.0 μm and contain polar tubules with four to seven coils in single rows (31).

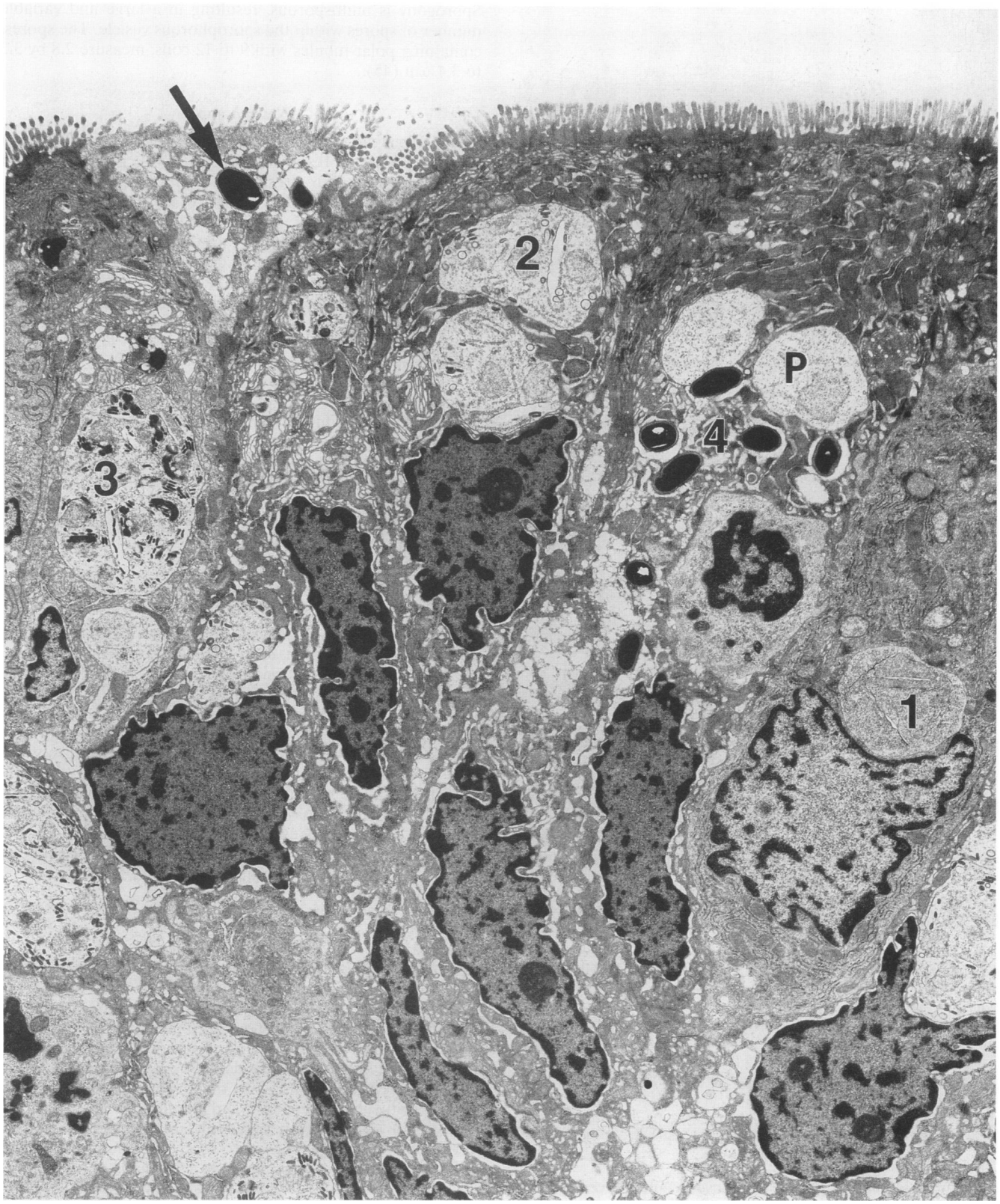


FIG. 5. Transmission electron micrograph showing jejunal villus epithelium heavily parasitized with *Enterocytozoon bieneusi* organisms between the enterocyte nuclei and the microvillous border. Stages include the following: (1) a proliferative plasmodium, which is depressing and indenting the host cell nucleus; (2) early sporogonial plasmodia, containing characteristic electron-lucent inclusions; (3) late sporogonial plasmodia, containing developing spores, each with a polar tubule; and (4) mature condensed spores in an enterocyte also parasitized by immature plasmodia (P). A mature spore (arrow) in a necrotic enterocyte is positioned to enter the lumen when its host enterocyte desquamates. Magnification, ×5,657. From reference 36, with permission of the publisher.

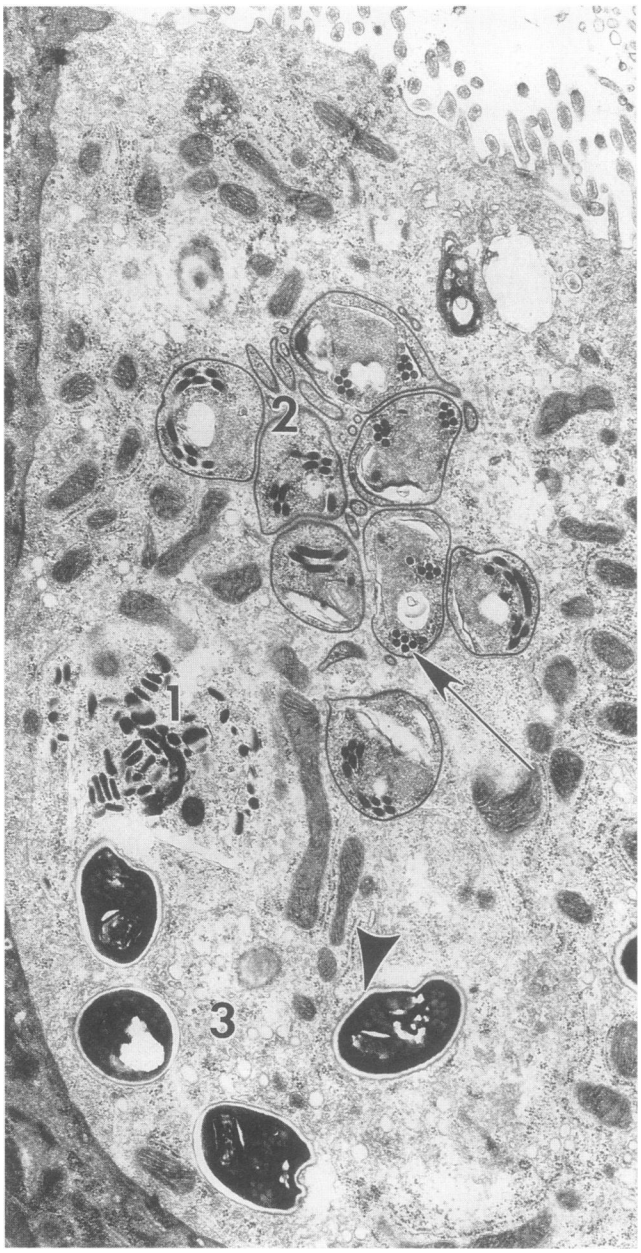


FIG. 6. Transmission electron micrograph depicting an enterocyte containing (1) sporogonial plasmodium of *Enterocytozoon bieneusi*, (2) several newly formed sporoblasts, and (3) mature condensed spores, each surrounded by an electron-lucent endospore layer and an outer electron-dense exospore coat (arrowhead). The sporoblasts are irregular in shape, possess a thickened plasmalemma, and are sectioned at various angles through the electron-dense polar tubules which form five to six coils, arranged in two rows (arrow). Magnification, ×13,520. From reference 36, with permission of the publisher.

Pleistophora sp.

Pleistophora sp. is parasitic in insects and in vertebrates, mainly fish (39, 45). In humans, it has been identified in the muscles of immunodeficient patients with myositis (51, 116).

The parasite develops intracellularly within a vesicle bounded by a thick amorphous parasite-formed coat, termed sporophorous vesicle. Nuclei of all developmental stages are unpaired. Merogonic proliferation forms multinucleate plasmodia. Sporogony is multisporous, resulting in a large and variable number of spores within the sporophorous vesicle. The spores, containing polar tubules with 9 to 12 coils, measure 2.8 by 3.2 to 3.4 μm (45).

Nosema spp.

Numerous *Nosema* species which are parasitic primarily in invertebrates have been described (39, 45). Development of the parasite occurs in direct contact with host cell cytoplasm, and nuclei are paired (diplokaryotic) (45).

One case of disseminated *Nosema* infection in an athymic child has been reported and involved almost all tissues examined at autopsy (125). Not all developmental stages of the parasite could be documented, and only sporoblasts with immature and mature spores were found. The spores were diplokaryotic and measured 2.0 to 2.5 by 4.0 to 4.5 μm, containing polar tubules with approximately 11 coils. The parasite was named *Nosema connori* (125, 199).

Two cases of keratitis due to *Nosema* spp. have been observed in otherwise healthy persons. In one patient, parasites were identified in deep corneal stroma and were isolated by cell culture. The spores, containing polar tubules with six coils, were diplokaryotic and measured 1.0 by 3.7 μm. The organism was named *Nosema corneum* (61, 193). In a second patient, diplokaryotic spores, measuring 3 by 5 μm and containing polar tubules with 11 to 12 coils, were found in biopsy samples from a corneal ulcer. The parasite was named *Nosema ocularum* (32).

Genera Unknown

Microsporidium is a collective term for organisms that cannot be taxonomically classified because appropriate information is not available, especially because details of the life cycle of the parasites are missing (45).

Microsporidium ceylonensis was identified in a corneal ulcer of a boy from Sri Lanka. Spores measuring 1.5 by 3.5 μm were detected free in the corneal stroma (5). Meronts and sporonts were not seen, and nucleation was not observed. *Microsporidium africanum* was detected in the corneal stroma of a woman from Botswana suffering from a perforated corneal ulcer (161). Developmental stages of the parasite were not seen. Spores, measuring 2.5 by 4.5 μm, contained polar tubules with 11 to 13 coils (161). Immune status was unknown in both of these patients.

EPIDEMIOLOGY

Data on epidemiologic characteristics of human microsporidiosis are rapidly increasing, but many questions have yet to be answered. Intriguing information contributing to our understanding includes the expanding clinical spectrum of human disease; the prevalence data of ongoing prospective studies of HIV-associated microsporidiosis; the recognized potential for person-to-person transmission; the hypothesized absence of microsporidial host specificity; and the ubiquitousness of these parasites in nature, with seemingly infinite possibilities for environmental and animal reservoirs (25). The epidemiology of human microsporidiosis may vary according to host immune status and the infecting species of microsporidia (25, 42).

Because diagnosis of microsporidial infection is still dependent on morphological demonstration of the organisms, the strength of epidemiologic surveys is limited. The interpretation and usefulness of the scant seroprevalence data available are controversial (25). With antigens obtained from cultures of murine-derived strains of *Encephalitozoon cuniculi*, serologic

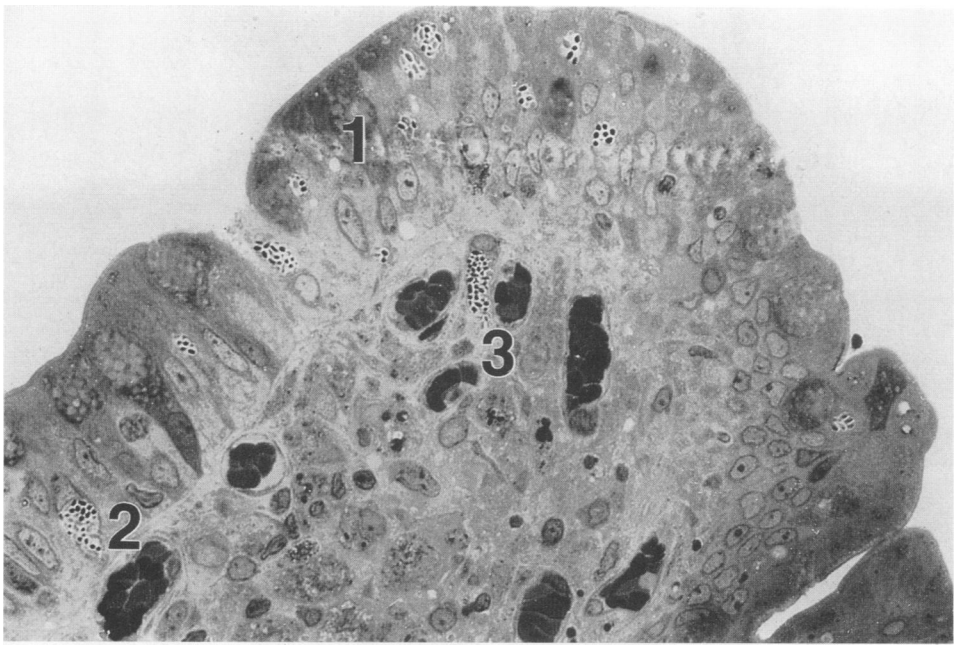


FIG. 7. Transmission electron micrograph showing the apex of a duodenal villus from a patient with AIDS infected with *S. intestinalis*. Spores are clustered in parasitophorous vacuoles which are located (1) between enterocyte nuclei and the microvillous border, (2) in basal positions below enterocyte nuclei, and (3) within cells in the lamina propria, below the epithelium. Compare with developmental stages of *Enterocytozoon bieneusi* in Fig. 5 and 9 which are located only above enterocyte nuclei, without invasion of the lamina propria. Magnification, ×175.

surveys for human antibodies to *Encephalitozoon cuniculi* performed in the 1980s suggested that travelers and residents in tropical countries may have increased exposure to this organism, but clinical correlation and definitive epidemiologic data were lacking (11, 12, 25, 41, 95, 98, 197, 246). In addition, recent findings suggest that serologic cross-reactivity to various microsporidial antigens complicates the interpretation of seroprevalence data (25, 69, 244).

Prevalence and Geographic Distribution

Several early reports of suspected cases of human microsporidial infection (54, 208–210, 243, 248–250) could not be confirmed, either because the type material had been lost or because reexamination established that the suspected etiologic agent was not a microsporidian (8, 243). An association between microsporidial infection and multiple sclerosis (49, 225) and the description of the occurrence of microsporidia in the cytoplasm of tumor cells of a pancreatic adenocarcinoma (124) could also not be substantiated (45).

Among persons not infected with HIV, 10 cases of microsporidial infection have been sufficiently documented (Table 1) (25, 26, 29, 42). Of note, intestinal *Enterocytozoon bieneusi* infection, which previously had exclusively been found in HIV-infected patients, has recently been observed in a healthy person with self-limiting diarrhea who had travelled in the Middle East (175) and in a patient who had undergone organ transplantation (176). Intestinal *Enterocytozoon bieneusi* infection has also been reported in African children with diarrhea who lived in an area of low HIV prevalence, but the HIV serostatus was not determined in these children (24). Canning has suggested that the common occurrence and widespread distribution of human *Enterocytozoon bieneusi* infections indicate that it may be a natural parasite of humans, transiently infecting the immunocompetent but causing disease only in the immunosuppressed (42).

In patients with HIV infection, four genera of microsporidia and over 400 cases of microsporidiosis have been documented (29), the majority attributed to *Enterocytozoon bieneusi* (2, 13–15, 19, 22, 24, 36, 40, 43, 52, 53, 60, 63, 66, 72, 74, 77–79, 83, 86, 87, 98, 108–110, 122, 133–135, 138, 139, 146, 148, 149, 152, 166, 169, 174, 179, 205, 212, 220, 221, 232, 236, 237, 240). Most clinical and epidemiologic studies indicate that *Enterocytozoon bieneusi* is consistently associated with intestinal and/or biliary illness and is present in approximately 7 to 50% of severely immunodeficient HIV-infected patients with CD4 cells below 100/ml^3 and otherwise unexplained chronic diarrhea (Table 4) (47, 63, 77, 78, 84, 87, 122, 134, 138, 147, 148, 220, 237, 240). *Enterocytozoon bieneusi* may also cause self-limiting diarrhea in HIV-infected persons, particularly when cellular immunodeficiency is less severe (i.e., when CD4 cells are above 100 to 200/ml^3) (233, 237). Some investigators have questioned the association between microsporidiosis and diarrhea because a case control study comparing intestinal biopsies of 55 HIV-infected patients with chronic diarrhea and 51 HIV-infected patients without diarrhea found no significant difference in the occurrence of microsporidiosis in the two patient groups (166). In one of the few studies to evaluate stool specimens from HIV-infected persons without diarrhea, 5 of 17 confirmed cases of intestinal microsporidiosis were in persons reporting no diarrheal symptoms, suggesting the possibility of asymptomatic enteric carriage (22). These findings, however, will require further evaluation as the majority of published reports show a strong correlation between the presence of organisms and diarrheal disease. Weber and colleagues, for example, examined 215 stool specimens from 134 HIV-infected persons in the United States and found no microsporidial spores in nondiarrheal specimens (232). Examination of 1,271 stool specimens from 845 HIV-infected persons in Switzerland revealed mi-

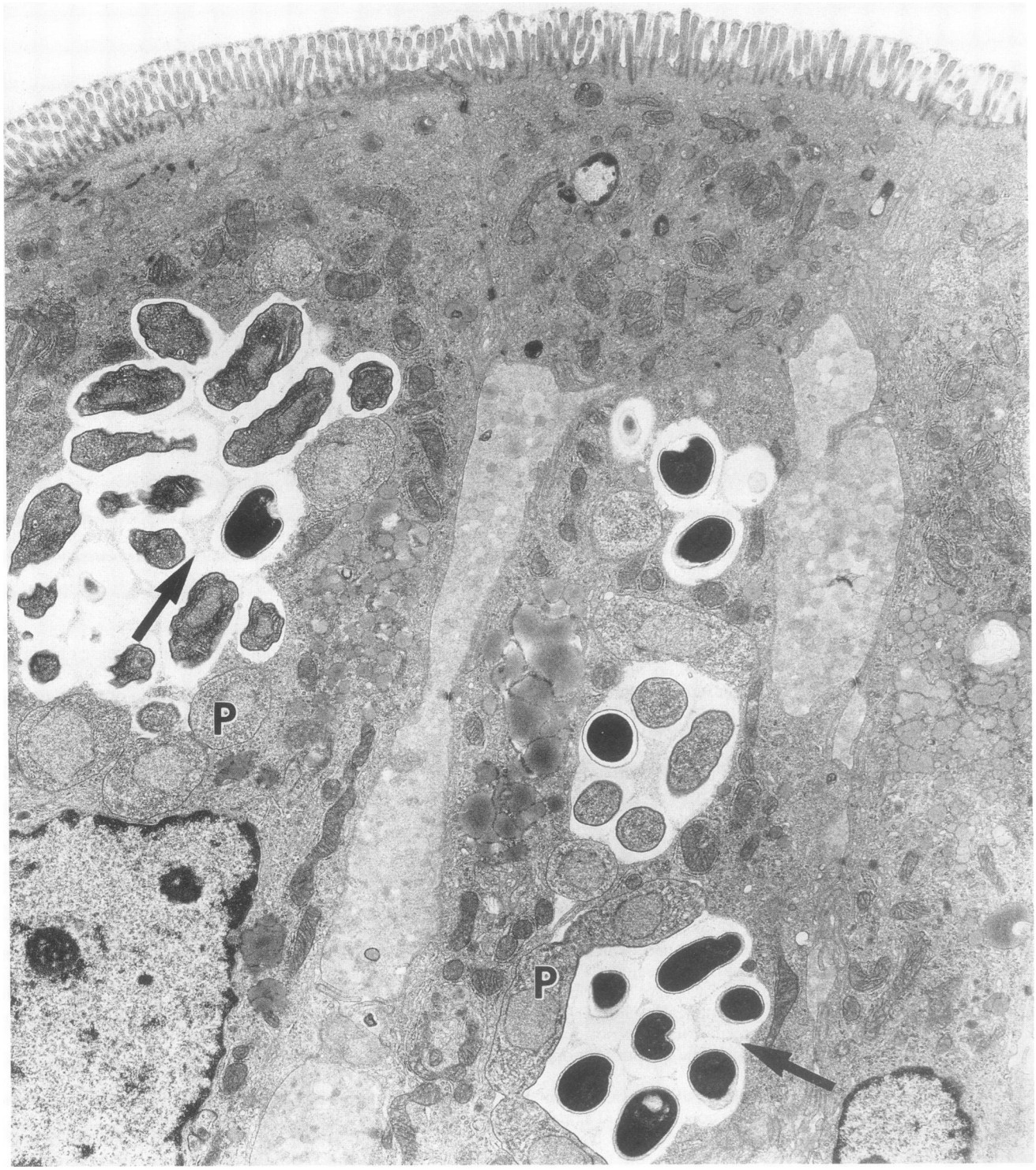


FIG. 8. Transmission electron micrograph of developing *S. intestinalis* spores within enterocytes separated by a fibrillar matrix (arrows), giving rise to the genus name *Septata*. Proliferative forms (P) develop asynchronously and surround the mature and maturing spores. *S. intestinalis* spores within parasitophorous vacuoles are segregated from enterocyte cytoplasm, unlike *Enterocytozoon bieneusi* spores, which lie in direct contact with enterocyte cytoplasm (cf. Fig. 5 and 6). Magnification, ×7,260.

crosporidial spores in 8 of 88 patients with chronic diarrhea and in 3 of 57 patients with self-limiting diarrhea (these patients continued to excrete microsporidial spores after cessation of diarrhea) and no spores in 700 asymptomatic patients (237). Nevertheless, coprodiagnostic techniques may not be sensitive enough to detect "low-level" asymptomatic infection.

Epidemiologic data on the prevalence of intestinal pathogens in HIV-infected patients with chronic diarrhea who had

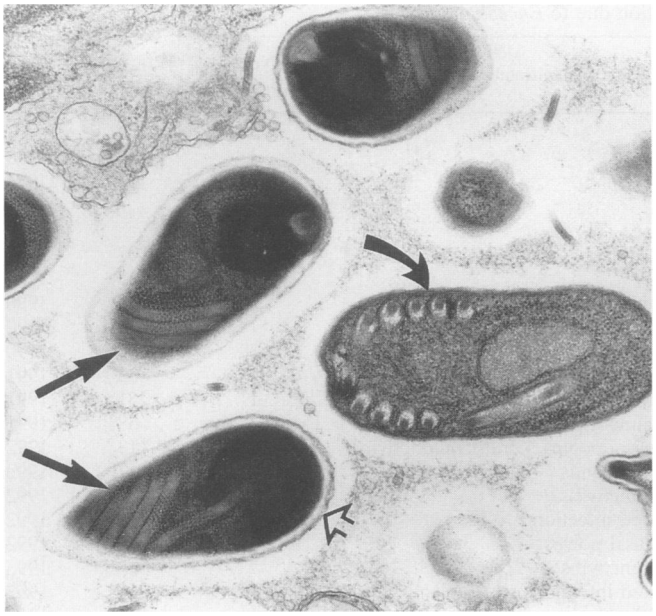


FIG. 9. Transmission electron micrograph of mature *S. intestinalis* spores. Spores contain polar tubules in spirals of four to seven coils (straight arrows), are coated by an electron-lucent endospore and an electron-dense exospore (arrowhead), and are surrounded by fibrillar septa. The polar tubules of *S. intestinalis* lie in single rows (curved arrow) at the spore periphery, which is in contrast to double rows of polar tubules seen in *Enterocytozoon bieneusi* spores (cf. Fig. 6). Magnification, ×21,250.

TABLE 4. Prevalence of *Enterocytozoon bieneusi* in HIV-infected patients with chronic diarrhea

Study type and locale	Prevalence (%)	No. of patients positive/no. examined	Reference	
			No.	Yr
Prospective studies including techniques to detect microsporidia in stool specimens				
Paris, France	16	31/198	63	1993
Amsterdam, The Netherlands	11	16/143	220	1993
Atlanta, Ga.	10	17/162	240	1993
Zurich, Switzerland	9	8/88	237	1993
Studies including endoscopic examination of patients with unexplained diarrhea[a]				
Paris, France	50	9/18	138	1993
Sydney, Australia	33	36/109	78	1993
Amsterdam, The Netherlands	27	16/55	77	1991
New York, N.Y.; Bethesda, Md.; Washington D.C.	26	100/379	148	1992
Baltimore, Md.	23	5/22	87	1991
London, United Kingdom	19	40/210	84	1992
Nice, France	16	13/81	134	1993
Multicenter study, United States	12	6/51	47	1991
Uganda and Zambia, Africa	7	5/77	122	1989

[a] These studies usually did not include techniques for detection of microsporidia in stool specimens.

endoscopic evaluation may overestimate the prevalence of microsporidiosis because of selection bias; i.e., it is assumed that primarily patients with the most severe diarrhea and negative routine stool examination would agree to undergo invasive diagnostic procedures. Indeed, ongoing prospective studies of HIV-associated chronic diarrhea indicate a lower but still substantial prevalence of intestinal microsporidiosis of 9 to 16% in patient groups who are evaluated by coprodiagnostic techniques to detect microsporidia (Table 4) (63, 220, 237, 240). It is unclear whether this range in prevalence represents true geographic variation, differences in the abilities of various investigators to identify microsporidian-infected patients, or differences in risk factors for exposure to microsporidia.

HIV-associated infections with other microsporidia have been observed less frequently. As of July 1993, we were aware of approximately 25 well-documented cases of human *Encephalitozoon* infection (29). In most cases, the parasite was first detected in corneal or conjunctival specimens of patients with keratoconjunctivitis. Yet, the spectrum of recognized *Encephalitozoon*-associated disease in patients with AIDS appears to be expanding and includes bronchiolitis, sinusitis, nephritis, cystitis or ureteritis, hepatitis, and peritonitis (25, 29) (Table 5). Another microsporidian, *S. intestinalis*, has recently been described in association with intestinal and disseminated infection in about 20 HIV-infected persons (2, 76, 78, 150, 153, 220, 231, 239). Among them, some patients infected with organisms consistent with *S. intestinalis* have been reported as being infected with *Encephalitozoon* sp. (78, 220). Myositis in a patient with AIDS due to *Pleistophora* sp. has been reported once (51).

Reported human infections are globally dispersed and have been documented in persons from all continents except Antarctica (29, 40, 78, 122, 139, 146).

Sources of Human Infection and Transmission

The sources of microsporidia infecting humans and modes of transmission are uncertain. As microsporidia are obligate intracellular parasites which are released into the environment via stool, urine, and respiratory secretions, possible sources of infection may be persons or animals infected with microsporidia. Whether human microsporidiosis may be a zoonosis is unknown. Epidemiologic research is limited, and animal reservoir hosts of microsporidia infecting humans have not been confirmed (25, 39, 42, 45). Also, intermediate hosts or invertebrate vectors of microsporidia have not been discovered in mammal or human microsporidiosis (39). Although *Encephalitozoon* sp. is commonly found in mammals and occasionally in birds (39, 45), an animal source of human encephalitozoonosis has not been demonstrated, and available data indicate that most *Encephalitozoon* species isolated from humans are distinct from *Encephalitozoon cuniculi* of animal origin (68, 70, 223). Nevertheless, new clues of potential animal reservoirs continue to emerge. Jafri and colleagues have evaluated fecal specimens obtained from dogs housed in a busy urban animal shelter. They found that 6 of 20 dogs were excreting microsporidial spores in their stools (103). Microsporidia have a wide zoological distribution, but arthropods are the most common hosts. Whether insect microsporidia might infect mammals or humans is unknown (191, 192, 214). Nevertheless, infection of a mouse by a mosquito microsporidial parasite (*Nosema algerae*) has been achieved experimentally (215), but another insect microsporidian did not replicate in mice (192). The presence of spores in surface water samples also suggests the possibility of environmentally acquired infection, but no human-infecting microsporidia have yet been identified from such sources (6).

Ingestion of spores, which had been released into the environment, is a plausible mode of transmission of the human

TABLE 5. Synopsis of human infection due to *Encephalitozoon* spp.

Study population and country	Species	Clinical manifestation(s)	No. of patients	Reference	
				No.	Yr
Children without HIV infection					
Japan	*E. cuniculi*	Seizures	1	127	1959
Sweden	*E. cuniculi*	Seizures	1	11	1984
HIV-infected patients					
United States	*E. cuniculi*	Hepatitis	1	207	1987
Switzerland	*E. cuniculi*	Peritonitis	1	253	1989
United States	*Encephalitozoon*[a]	Keratoconjunctivitis	5[b]	48	1990
United States	*Encephalitozoon*[a]	Keratoconjunctivitis	1[b]	121	1990
United States	*Encephalitozoon*[a]	Keratoconjunctivitis	3[b]	80	1990
United States	*Encephalitozoon*[a]	Keratoconjunctivitis	1	33	1991
United States	*Encephalitozoon*[a]	Keratoconjunctivitis	1[b]	251	1991
United States	*E. hellem*	Keratoconjunctivitis	3[c]	68	1991
United Kingdom	*Encephalitozoon*[a]	Sinusitis, nasal polyps, keratoconjunctivitis	1	113	1992
Canada	*Encephalitozoon*[a]	Keratoconjunctivitis	1	67	1992
United Kingdom	*Encephalitozoon*[a]	Keratoconjunctivitis	1	132	1992
United States	*E. hellem*	Disseminated infection	1[d]	180	1992
The Netherlands	*Encephalitozoon*[a]	Sinusitis, nasal polyps	1	76	1992
United States-Switzerland	*E. hellem*	Keratoconjunctivitis	7	183	1993
Switzerland	*E. hellem*	Disseminated infection	1[d]	235	1993
United States	*E. hellem*	Bronchiolitis, pneumonia	1[d]	184	1993
United Kingdom	*Encephalitozoon*[a]	Nephritis	1	97	1993
United States	*E. cuniculi*	Disseminated infection	1	62	1994

[a] Species not designated.
[b] Patients reported in the *Morbidity and Mortality Weekly Report* have been described more comprehensively in references 80, 121, and 251.
[c] One patient is identical to a patient described in reference 80.
[d] These patients are also included in reference 183.

species *Enterocytozoon bieneusi* and *S. intestinalis*. Both parasites develop mainly in small-intestinal enterocytes and are excreted with feces. *S. intestinalis* is also disseminated to the kidney and appears to be shed in urine (2, 150, 231). Potential person-to-person transmission may be considered because both species have been identified only in humans so far. In human *Encephalitozoon* infection, spores have been found in urine, sputum, and conjunctival fluid, but they have not been detected in fecal material (25, 29, 235). *Encephalitozoon* infection in animals by the oral route has been documented experimentally (55, 57).

A possible transmission of microsporidia by the aerosol route has also been discussed because spores have been found repeatedly in the sputum of patients with *Encephalitozoon hellem* infection (62, 184, 224, 235). Moreover, *Enterocytozoon bieneusi*, *Encephalitozoon hellem*, and *S. intestinalis* have been detected in bronchial epithelial tissue and bronchoalveolar lavage specimens (62, 150, 180, 182, 184, 185, 234), as well as in nasal discharge and nasal epithelial tissue (38, 76, 79, 90, 182). Experimentally, pulmonary infections have been induced by instillation of *Encephalitozoon* spores into the nasal cavities as well as by intratracheal injection of spores into rabbits, although the possibility cannot be excluded that spores were ingested by the animals during these experiments, and pulmonary infection occurred secondary to dissemination from the intestine (57). Keratoconjunctival infection might occur by direct inoculation of spores by contaminated fingers or by the aerosol route.

Animal experiments have suggested that rectal infection with *Encephalitozoon* analogous to sexual transmission of other protozoa (159, 165), may lead to disseminated microsporidiosis (247). Also, the extensive urinary tract involvement, including infection of the prostate, seen with human *Encephalitozoon hellem* infections raises the possibility of sexual transmission (186).

Epidemiologic observations and experimental studies in animals suggest that *Encephalitozoon* spp. can be transmitted transplacentally from mother to offspring (100, 252). Transplacental transmission may even be important in the pathogenesis of the disease, especially in foxes, dogs, and nonhuman primates such as squirrel monkeys (137, 145, 252). No congenitally acquired human infections have been reported.

PATHOLOGY AND PATHOGENESIS

Host-Parasite Interaction

The pathophysiology of human microsporidiosis is not fully understood. Because members of the genus *Encephalitozoon* are parasitic in human and nonhuman hosts, experimental and natural mammalian encephalitozoonosis may serve as an appropriate model to study systemic human microsporidiosis. Nevertheless, the disease encephalitozoonosis appears to vary markedly in relation to the host species, mode of infection, host factors such as age at time of infection, quality of the immune response, and possibly strain of parasite. No animal model exists for the species *Enterocytozoon bieneusi*.

Four categories of host-parasite interaction have been described for mammalian and human encephalitozoonosis (192): (i) latent asymptomatic or chronic mildly symptomatic infection, known in mice and rabbits of all ages and in dogs, foxes, and squirrel monkeys infected as adults (16, 45, 101); (ii) acute, potentially fatal disease, observed in neonatal dogs, foxes, and squirrel monkeys (21, 136, 137, 252); (iii) proliferation of the parasite in the absence of competent host defenses, seen in patients with cellular immunodeficiency and athymic mice in vivo and in cell cultures in vitro (81, 177); and (iv) symptomatic human disease in the immunocompetent host (175). Some studies indicate that clinical encephalitozoonosis in the animal

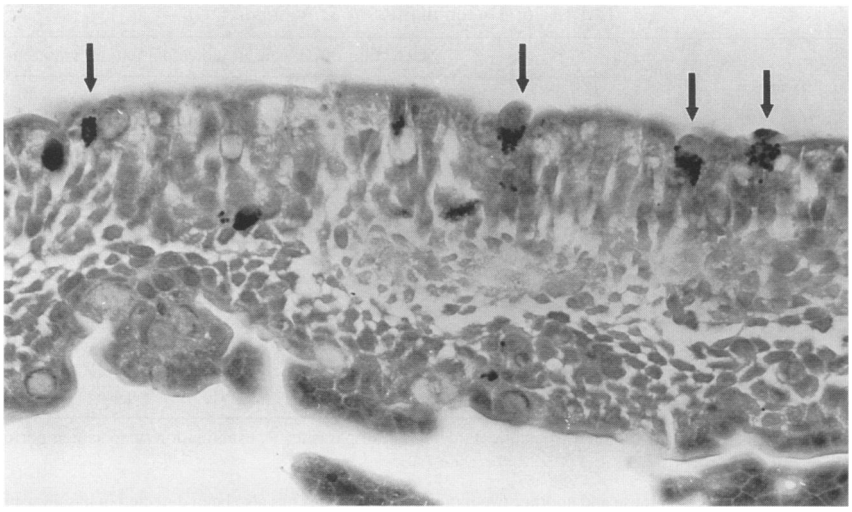


FIG. 10. Jejunal mucosal biopsy from a patient with AIDS and chronic diarrhea, demonstrating several epithelial cells (arrows) containing gram-positive spores of *Enterocytozoon bieneusi*. Tissue Gram stain (Brown & Hopps) was used. Magnification, ×200.

groups in category ii appears to be limited in many cases to transplacentally infected animals (39, 252).

Furthermore, animal experiments performed in the 1930s and 1960s have suggested that latent encephalitozoonosis in mice might enhance host resistance to experimentally induced tumors and that some tumor cells contaminated with *Encephalitozoon* spp. might have altered biological behavior (4, 158, 211). More recent experiments have indicated that nonspecific resistance to infectious agents other than microsporidia may be increased or depressed by latent encephalitozoonosis (56, 194). Also, alteration of more specific immunologic responses, including enhanced killer cell activity and activation of macrophages, has been demonstrated in animals with encephalitozoonosis (142).

In humans, different host-parasite interactions may be observed depending on the microsporidial species and the competence of the immune response. In immunocompetent and otherwise healthy persons, acute intestinal, self-limiting microsporidiosis may occur (175), but systemic microsporidiosis has not been satisfactorily documented in a previously healthy person (Table 1). Epidemiologic data suggest that patients with severe cellular immunodeficiency are at highest risk for developing microsporidial disease. It is not understood, however, whether microsporidiosis in these immunodeficient patients is primarily a reactivation of latent infection acquired prior to the state of suppressed immunity or whether microsporidial disease is caused by recently acquired infection.

Lesions and Dissemination of the Parasite

Aside from studies of ocular microsporidiosis in presumably otherwise healthy persons, detailed histopathologic investigation of human microsporidial infection has been performed only in immunodeficient individuals. In these patients, inflammatory reaction in tissue infected with microsporidia is often minimal or even absent (147). In contrast, inflammatory reaction in animal encephalitozoonosis is typically an intense diffuse cellular infiltration or granulomatous lesion characterized by infiltrations of mononuclear cells, including lymphocytes, plasma cells, and macrophages, often around a necrotic center. These lesions may persist after the disappearance of the organisms themselves (45, 81, 115, 216, 217).

Intestinal *Enterocytozoon bieneusi* infection. Human *E. bieneusi* infections generally appear to be limited to intestinal and biliary epithelium. Infections begin in small-intestinal enterocytes in which the parasites are located in the supranuclear area of the apical cytoplasm (Fig. 10 and see Fig. 17H). Histologic findings may range from virtually normal villus architecture to severe epithelial degeneration (77, 87, 110, 135, 138, 147, 149, 157, 232). In some cases, histopathologic changes have included villus atrophy and fusion, crypt elongation, goblet cell depletion, prominent intraepithelial lymphocytic infiltrates, and enterocyte vesiculation, swelling, or sloughing. In other cases, general preservation of villus architecture in association with minimal (or absent) lamina propria mononuclear cell infiltrates has been noted (77, 87, 110, 135, 138, 147, 149, 157, 232). *E. bieneusi* infection may be accompanied by alterations in small-bowel physiology, comparable to findings in patients with the so-called AIDS enteropathy (213), such as decreased brush border disaccharidase-specific enzyme activities (108, 110).

E. bieneusi has also been identified in biliary epithelium (10, 131, 163, 164), nonparenchymal liver cells (164), and pancreatic ductal epithelium (27), as well as in tracheal (148), bronchial (234), and nasal (76, 90) epithelia. These lining epithelia generally appear undamaged, and intraepithelial infiltration by lymphocytes is minimal. Infections of the biliary tract may be associated with papillary stenosis, bile duct dilatation, acalculous cholecystitis, and sclerosing cholangitis (10, 131, 163, 164). Biliary tract and pancreatic ductal microsporidiosis may reflect per continuitatem spread of infection, but it is not known whether microsporidial infection of the airways is acquired by the aerosol route, by aspiration, or by hematogenous dissemination from other sites (234). Histopathologic findings of *E. bieneusi* in the subepithelium and lamina propria in one patient might indicate that dissemination of *E. bieneusi* rarely may occur (188).

Intestinal and disseminated *S. intestinalis* infection. *S. intestinalis* also infects primarily small-intestinal enterocytes. Inflammatory response as well as intestinal cell injury are similarly minimal, but infection does not remain confined to epithelial cells (Fig. 7). *S. intestinalis* is also found in the perinuclear zone of intestinal lamina propria macrophages and in fibroblasts and endothelial cells. Dissemination to kidneys, lower airways, and biliary tract appears to occur via infected

TABLE 6. Dissemination of *S. intestinalis*

Method of detection and specimen	Detection of parasite in individual patient (reference[s])[a]:									
	1 (150, 153)	2 (150)	3 (150)[b]	4 (150)	5 (150)[c]	6–9 (78)[d]	10 (168)	11 (231)	12 (239)	13 (239)
Histological examination										
Small-intestinal enterocytes	+	+	+	+	−	+	ND	+	+	ND
Macrophages (intestinal lamina propria)	+	+	+	−	−	+	ND	+	+	ND
Bile duct and gallbladder	ND	ND	+	ND	−	ND	ND	ND	ND	ND
Nonparenchymal liver cells	ND	ND	ND	ND	+	ND	ND	ND	ND	ND
Bronchial epithelium	ND	ND	ND	ND	+	ND	ND	ND	ND	ND
Kidney	ND	ND	ND	ND	+	ND	ND	ND	ND	ND
Bodily fluids										
Urine	ND	+	+	−	ND	+ (4/4)	ND	+	−	−
Stool	ND	+	−	+	ND	+ (4/4)	+	+	+	+

[a] ND, examination not done or not reported or specimen not available; +, detection of parasite; −, examination of specimen performed, no detection of parasite.
[b] Patient had cholecystectomy.
[c] Autopsy material.
[d] Preliminary follow-up data have been reported on these and another two patients. *S. intestinalis* has also been detected in nasal washings, sputum, and tissue sections of nasal epithelium in some patients (79, 92).

macrophages (Table 6). Histopathologic findings of kidneys show granulomatous tubulointerstitial nephritis with inflammatory infiltrates, including macrophages, lymphocytes, plasma cells, and Langhans-type multinucleated giant cells (31, 150, 153).

Disseminated encephalitozoonosis. In animal *Encephalitozoon* microsporidiosis, the primary site of infection is usually the small-intestinal enterocyte from which the parasites are dispersed to various other organs. In the mouse, the first extraintestinal site to show signs of infection is the liver. Subsequently, central nervous system vasculitis and interstitial nephritis are the predominant lesions. Yet, as a result of parasite infection of endothelial cells and macrophages, the organisms may also disseminate to most other tissues, including liver, spleen, suprarenal glands, pancreas, lungs, myocardium, and placenta (39, 45, 136, 137, 145, 204, 216, 217, 252).

The human species, *Encephalitozoon hellem*, has not been identified in the intestine. The parasite has been well documented to infect the epithelial surfaces of ocular, respiratory (including sinus), and urinary tissues, but the means by which initial infection is acquired and subsequent dissemination occurs has been unclear (25, 68, 180, 183–185, 223, 235).

Autopsy findings of a first case of systemic encephalitozoonosis in a patient with AIDS reported by Schwartz and colleagues, however, suggest that primary infection with *Encephalitozoon hellem* may be acquired via the respiratory tract (180). Parasites were found within numerous respiratory epithelial lining cells extending from the proximal trachea distally to small-order conducting airways, and the organisms decreased in frequency with decreasing airway diameter (Fig. 11). Small numbers of microsporidia were also observed in stromal cells within subepithelial granulation tissue in the trachea, suggesting a potential means for access to blood vessels and subsequent hematogenous dissemination to distant organs such as the kidneys (Fig. 12). Indeed, spores were also present within the lumens of smaller-order renal blood vessels.

Renal involvement in this case was extensive (Fig. 13 to 16). Autopsy findings have revealed diffuse interstitial nephritis with infiltrates consisting primarily of lymphocytes and plasma cells, with lesser number of macrophages and neutrophils. There was extensive tubular necrosis but no significant glomerular pathology. The parasites were also detected within degenerating tubules and necrotic areas. Ureters showed a granulomatous and lymphoplasmacytic inflammation, and ulcerating cystitis with lymphohistiocytic inflammatory reaction was present (180).

Liver infection due to *Encephalitozoon* sp. has been described in a single case report of an HIV-infected patient. Histologic examination showed granulomatous liver necrosis and the presence of parasites within vacuoles of the hepatic cells (207). Another single case report demonstrated a nongranulomatous inflammatory reaction and *Encephalitozoon*

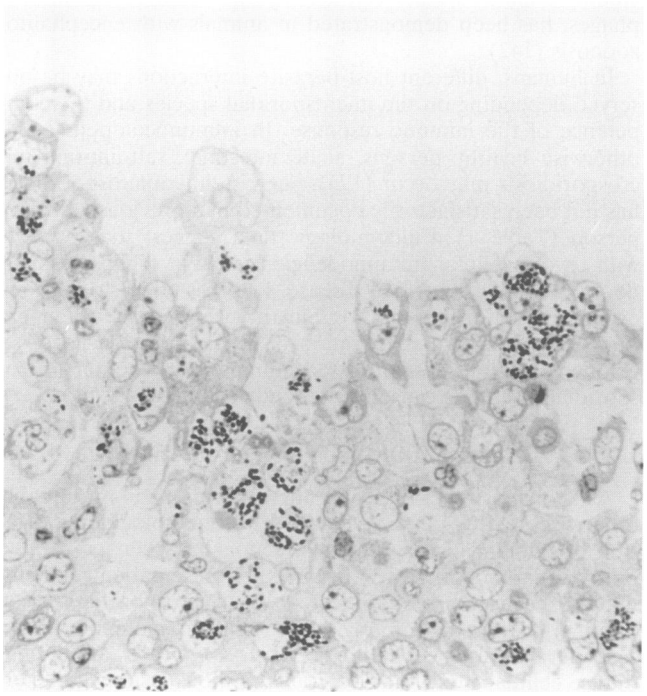


FIG. 11. Plastic-embedded semithin (1-μm) section of a major bronchus from a patient with disseminated *Encephalitozoon hellem* infection. The microsporidial spores are easily visualized within the cytoplasm of the lining epithelium. Toluidine blue stain was used. Magnification, ×400.

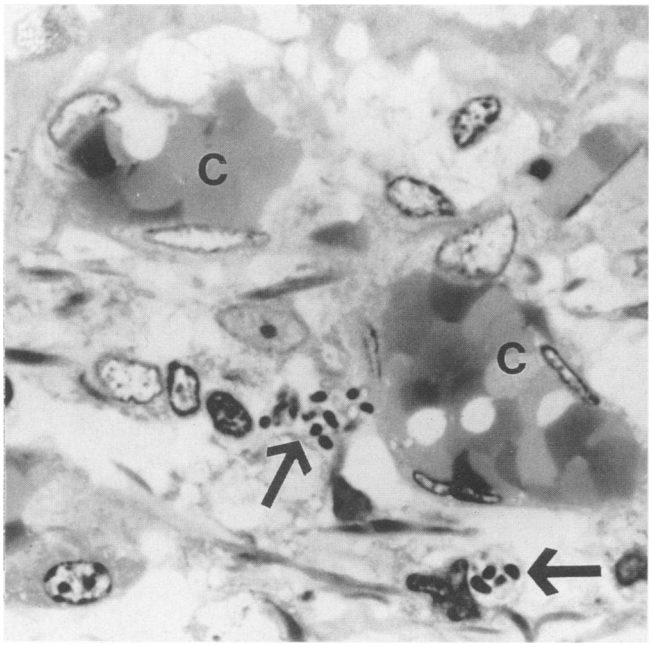


FIG. 12. Plastic-embedded semithin (1-μm) section of tracheal submucosa from a patient with AIDS and disseminated *Encephalitozoon hellem* infection showing spores of *E. hellem* (arrows) adjacent to capillaries (C) in granulation tissue. Toluidine blue stain was used. Magnification, ×400.

organisms in the omentum magnum of a patient with AIDS and peritonitis (253). Although reported as *Encephalitozoon cuniculi*, available information is inadequate to determine whether these organisms were truly *Encephalitozoon cuniculi* or whether they were *Encephalitozoon hellem* or *S. intestinalis*.

Systemic *Nosema* infection. Autopsy findings in the case of a 4-month-old athymic child demonstrated parasites disseminated to all organs examined except the spleen (125). The central nervous system was not examined. The parasite load was highest in the diaphragm, in all layers of the muscularis of the gastrointestinal tract, and in smooth-muscle cells of arteries in the urinary bladder, kidney, liver, adrenals, heart, and diaphragm, but parasites were also found in parenchymal cells of the lung, liver, kidney, the adrenal cortex, and myocardium. The high parasite load contrasted with a minimal inflammatory reaction in these organ systems. The presence of most numerous organisms within intestinal tunica muscularis and the media of muscular arteries in several organs led the authors to postulate that their patient's infection began in the intestinal epithelium, with subsequent hematogenous dissemination (125).

Myositis. Histologic examination of muscle biopsies of two patients with myositis due to *Pleistophora* sp. showed atrophic and degenerating muscle fibers infiltrated by clusters of parasites. Whereas inflammatory reaction in the case of the HIV-seronegative patient with cellular immunodeficiency was intense, including plasma cells, lymphocytes, and histiocytes, inflammatory infiltrates were mild in the patient with AIDS (51, 116). The route of initial infection or the means of dissemination has not been established for either case.

Ocular infections. Ocular microsporidial infection has been classified pathologically as either stromal or epithelial, and its pathogenesis varies according to the immune status of the patient (25, 26, 32, 183). In otherwise healthy patients without immunodeficiency, ocular microsporidiosis was associated with keratitis or corneal ulcer, in some patients possibly related to prior trauma. The parasites involved, *Nosema* sp. and *Nosema*-like organisms of the nontaxonomic group *Microsporidium*, were found deep in the corneal stroma with spores contained within phagocytic cells and lying free between the fibrous

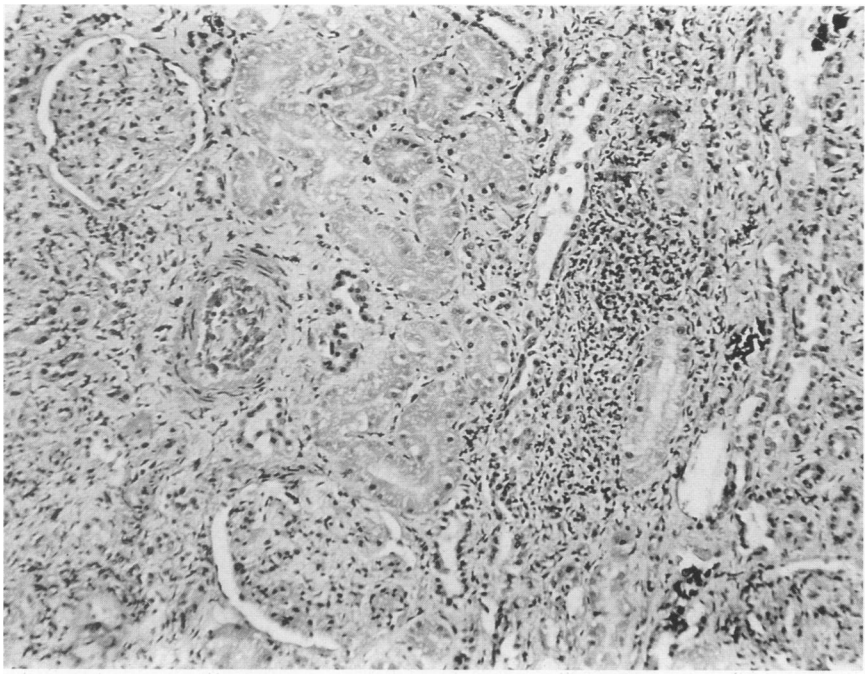


FIG. 13. Paraffin-embedded section of the kidney of a patient with disseminated *Encephalitozoon hellem* infection showing chronic and granulomatous interstitial nephritis and tubular necrosis. The microsporidia are not easily seen with routine hematoxylin-and-eosin staining. This patient excreted large numbers of spores in the urine. The section was stained with hematoxylin and eosin. Magnification, ×200.

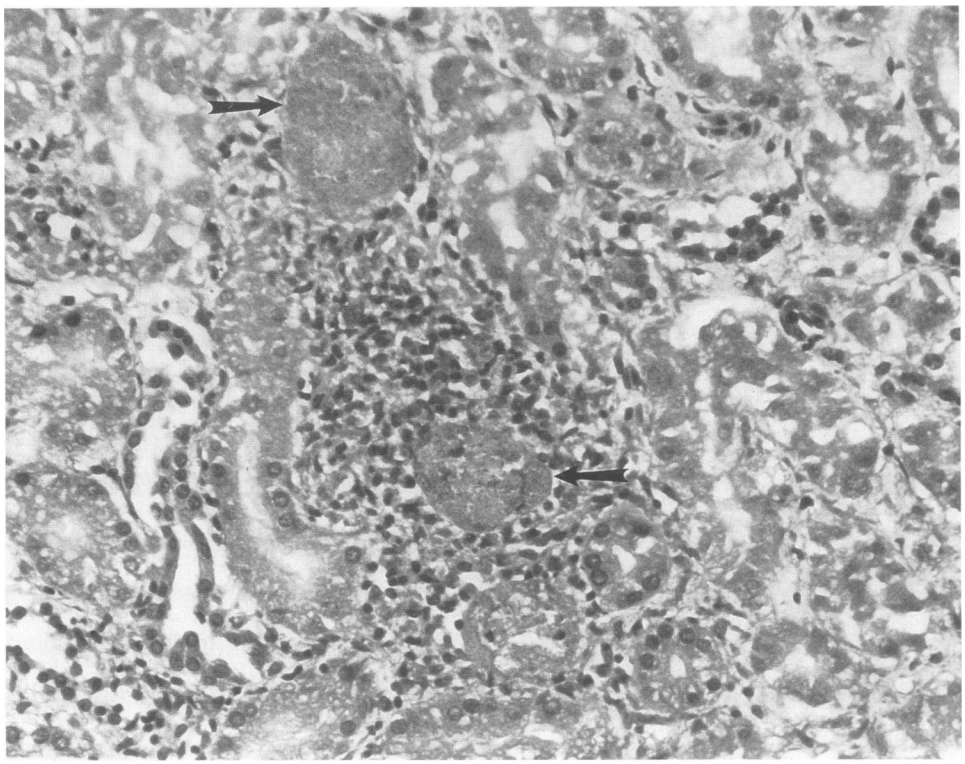


FIG. 14. Paraffin-embedded section of the kidney obtained following autopsy of a patient with AIDS and disseminated microsporidiosis depicting necrotic renal tubules filled with spores of *Encephalitozoon hellem* (arrows). The organisms appear as finely granular material when hematoxylin-and-eosin stains are used, and Gram stains are necessary to confirm microsporidial infection. The section was stained with hematoxylin and eosin. Magnification, ×400.

layers of corneal lamellae, and a marked inflammatory reaction, including mononuclear, neutrophil, and epitheloid infiltration, was present (5, 25, 26, 32, 61, 161, 193). No systemic microsporidial infection was noted in any of these patients.

In HIV-infected patients, in contrast, microsporidial parasites, *Encephalitozoon hellem* or other *Encephalitozoon*-like organisms, were confined to the superficial epithelial cells of the conjunctiva or cornea, associated with an inflammatory infiltrate composed mostly of neutrophils and mononuclear cells (26, 32, 33, 48, 67, 68, 80, 113, 121, 132, 180, 183, 235, 251). The inflammatory reaction was generally mild or even absent. As discussed below, these ocular infections occur primarily, if not exclusively, in patients systemically infected with *Encephalitozoon* spp. (25, 183).

Humoral and Cellular Immune Responses

Encephalitozoon cuniculi is able to persist in its animal host despite an active immune response. Latent infection remains asymptomatic as long as parasite multiplication and host immune response are balanced (81, 177, 178).

In animals, microsporidial infection activates antibody production, and persistence of antibodies reflects latent infection with the parasite. Antibodies alone, however, do not appear to provide protection, but some antibodies in mice have been found to have an opsonizing effect in vitro, which enables macrophages to kill the parasites (81, 143, 177, 178). The role of a competent cellular immune response in suppressing microsporidial multiplication has been established experimentally. Athymic mice infected with *Encephalitozoon cuniculi* die of the disease, but they can be protected by T-cell transfers from sensitized euthymic donor mice (177, 178). In contrast, immune serum from sensitized euthymic mice transferred to athymic mice does not stop or delay the progression of the *Encephalitozoon cuniculi* infection (81, 177). Corticosteroid treatment may reactivate latent microsporidial infection in mice (101).

In human microsporidiosis, the cellular immune response is evidently critical for preventing symptomatic microsporidial disease, which is predominantly associated with CD4 cellular deficiency. Some authors suggest that species such as *Enterocytozoon bieneusi* may be natural parasites of humans, possibly causing a transient diarrhea but normally remaining below the threshold of detection (42). Progression of cellular immunodeficiency, as observed in HIV infection, may reactivate latent microsporidial infections. The humoral response in human microsporidiosis has not been well elucidated, but analogous to other human opportunistic protozoal infections and experimental studies, microsporidian-specific antibodies alone may not be protective.

CLINICAL MANIFESTATIONS

The spectrum of clinically manifest microsporidial infection includes intestinal, ocular, muscular, and systemic disease (Tables 1 and 2). The most prevalent microsporidian-associated disease in HIV-infected patients is chronic diarrhea with wasting syndrome, but disseminated infection is increasingly recognized (Tables 1, 4, and 5).

In patients not infected with HIV, microsporidiosis may be classified into three patterns of disease (Table 1). (i) The first pattern is systemic microsporidiosis in patients with cellular

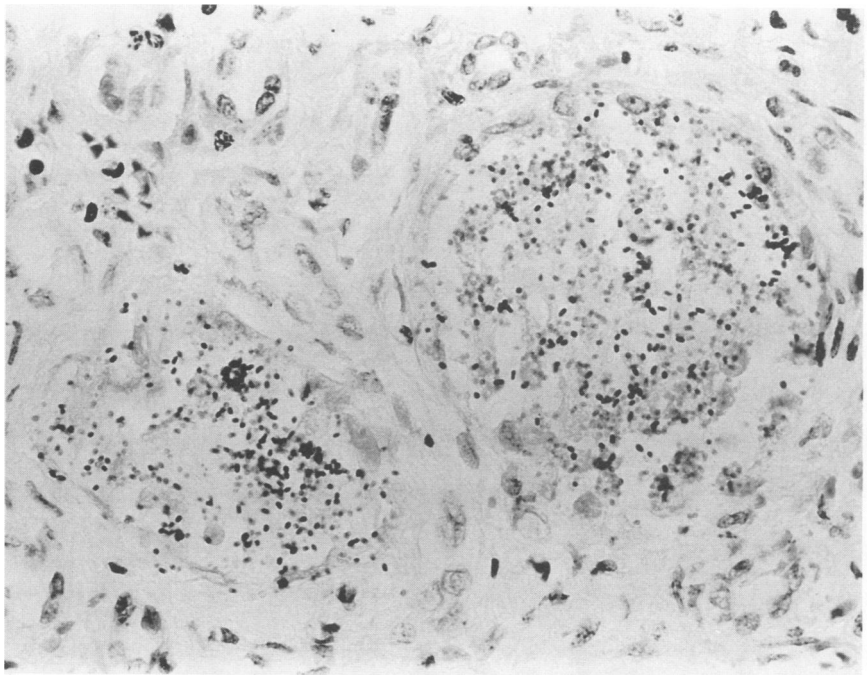


FIG. 15. Gram-stained, paraffin-embedded section of kidney showing two renal tubules filled with spores of *Encephalitozoon hellem*. The high parasite burden that can be present in infected tissues is clearly evident. Tissue Gram stain (Brown & Hopps) was used. Magnification, ×400.

immunodeficiency other than AIDS: three of the four HIV-seronegative patients described with systemic or muscular microsporidiosis had documented cellular immunodeficiency. The fourth patient may have had impaired immune response as indicated by cutaneous anergy to tuberculin antigen after *Mycobacterium bovis* BCG vaccination. (ii) The second disease pattern is corneal stroma infection in immunocompetent and otherwise healthy patients: the cornea may be considered an immunoprivileged site, because local immune response to infection in the cornea may be less intense compared with systemic inflammatory reaction. (iii) Intestinal microsporidiosis causing acute self-limiting diarrhea in immunocompetent persons, or patients with immunodeficiency other than AIDS, makes up the third pattern: two cases have been documented (175, 176).

Systemic Microsporidiosis Not Associated with HIV Infection

***Encephalitozoon* spp.** Two cases of children with seizure disorders attributed to *Encephalitozoon* infection have been described. In 1959, a 9-year-old Japanese boy presented with recurrent fever, loss of consciousness, headache, vomiting, and spastic convulsions. Organisms consistent with *Encephalitozoon* spp. were found in cerebrospinal fluid and urine. The patient had no renal symptoms, and urinalysis was normal. He was treated with penicillin and sulfisoxazole and recovered (127). In 1984, a 2-year-old Colombian child, domiciled in Sweden, with a low ratio of CD4 to CD8 lymphocytes had two convulsive seizures. Computed tomography of the brain was normal. Gram-positive organisms consistent with *Encephalitozoon* spp. were identified in urine specimens, and antibodies (both immunoglobulins M and G) to *Encephalitozoon cuniculi* were found in serum specimens. Urinalysis, blood urea nitrogen, and intravenous pyelography were within normal limits (11).

N. connori. Disseminated *N. connori* infection and *Pneumocystis carinii* pneumonia were diagnosed at autopsy in a 4-month-old athymic male infant reported in 1973 (125). Shortly after birth, the illness began with diarrhea, which became chronic. The child then developed vomiting, fever, dyspnea, weight loss, and mechanical ileus. Laparotomy and various antibiotics failed to alter the deteriorating course.

TABLE 7. Ocular microsporidiosis: microsporidial species and clinical manifestations

Microsporidial species	Immunodeficiency present	Corneal stroma infection	Keratoconjunctivitis
Encephalitozoon hellem	Yes	No	Yes
Encephalitozoon sp.	Yes	No	Yes
Nosema corneum	No	Yes	Yes
Nosema ocularum	No	Yes	Yes
Microsporidium ceylonensis	No	Yes	Yes
Microsporidium africanum	No	Yes	Yes

Microsporidial Myositis

Myositis due to *Pleistophora* sp. has been described in an HIV-seronegative and in an HIV-infected patient, both of whom had severe cellular immunodeficiency. The 20-year-old HIV-seronegative male from Florida, reported in 1985, experienced progressive generalized muscle weakness over 7 months, fever, generalized lymphadenopathy, and weight loss. At admission, muscle enzyme levels were normal and the patient had no pain. He was treated with trimethoprim-sulfisoxazole. Four years later, the absence of anti-HIV antibodies was confirmed, but cellular immunodeficiency of unknown etiology was still present (116, 123). The 33-year-old HIV-infected Haitian man, reported in 1993, had typical presentation of myositis with muscle weakness, myalgias, and elevated creatine phosphokinase (2,914 U/liter). He developed

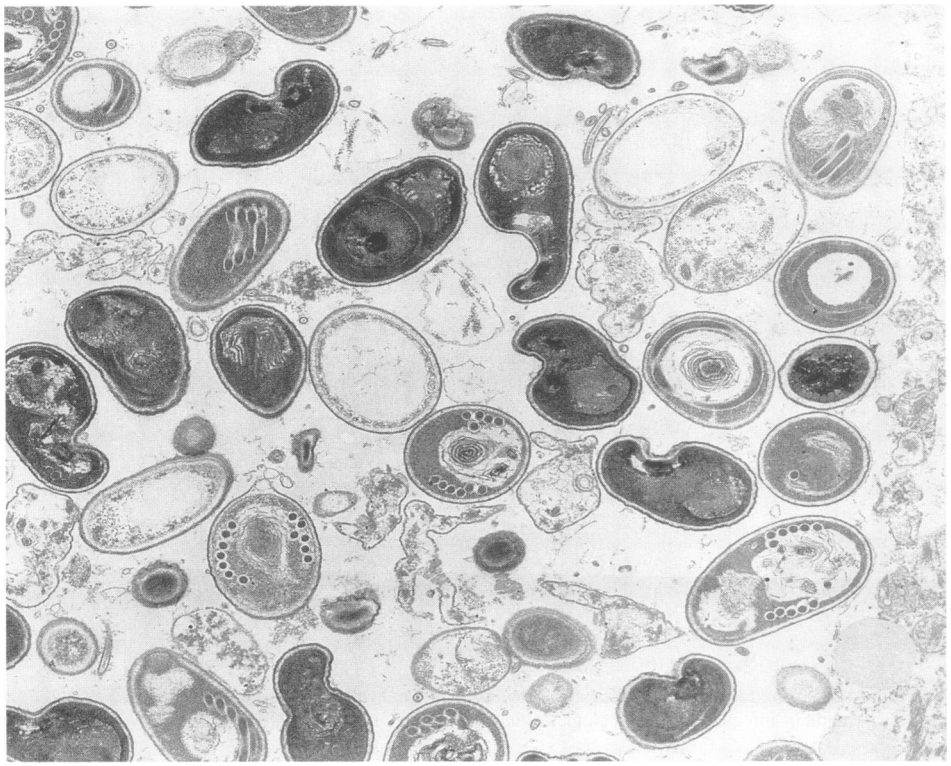


FIG. 16. Transmission electron micrograph of *Encephalitozoon hellem* infecting a renal tubular cell. Both mature thick-walled and immature thin-walled spores are present. The coiled polar tubules are easily seen. ×4,800.

nocardial pneumonia that was successfully treated, but myositis and cachexia progressed, and he died (51). In both patients, electromyogram and nerve conduction studies were consistent with a diffuse myopathy, and muscle biopsy was diagnostic.

Ocular Microsporidiosis

Corneal stroma infection in immunocompetent patients. Deep stromal infections of the cornea have been described in four otherwise seemingly healthy persons (Tables 1 and 7). They presented with severe keratitis, which had led to corneal ulcers in two of them.

In 1973, an 11-year-old boy from Sri Lanka presented with a severely scarred and vascularized right cornea; visual acuity was limited to light perception. Six years previously, he had been gored by a goat and required sutures in his right eyelid. Although visual acuity improved following keratoplasty, the graft eventually opacified. *Nosema*-like microsporidia (*M. ceylonensis*) were identified in corneal tissue (5, 45). In 1981, a 26-year-old woman from Botswana without a history of prior trauma sought medical attention for a painful left eye. Ophthalmologic examination revealed absence of light perception, conjunctivitis, and a central, perforated corneal ulcer with associated keratouveitis and hyphema. Topical therapy was unsuccessful, and enucleation of the left eye was performed. *Nosema*-like microsporidia (*M. africanum*) were detected in corneal stroma (45, 161).

In 1990, the case of a 45-year-old man from South Carolina was reported. Without prior trauma or contact lens wear, he developed decreased vision in his left eye during a 2-year course of progressive central disciform keratitis, recurrent anterior stromal infiltration with overlying punctate epitheliopathy, and anterior iritis. Topical prednisolone and antimicrobial agents were of no benefit, and diagnostic corneal biopsy was performed which revealed microsporidia (*N. corneum*). Decreasing visual acuity and an enlarging central disciform infiltrate led to the performance of a therapeutically successful keratoplasty (61, 193). The fourth patient, reported in 1991, was a 39-year-old man from Ohio who developed blurred vision and irritation of his left eye. Ophthalmologic examination revealed a corneal ulcer and a foreign body which was removed. The corneal ulcer, however, persisted, leading to the performance of a corneal biopsy which allowed the identification of microsporidia (*N. ocularum*). Topical therapy was of no benefit, and the patient eventually underwent successful corneal transplantation (26, 32).

Keratoconjunctivitis in HIV-infected patients. In HIV-infected patients, ocular microsporidial infection is restricted to the superficial epithelium of the cornea and conjunctiva (183). Recent data suggest that the species *Encephalitozoon hellem* is the most frequently identified etiologic pathogen (68, 183). Most patients exhibit bilateral coarse punctate epithelial keratopathy (Fig. 17A) and conjunctival inflammation, resulting in redness and foreign-body sensation, decreased visual acuity, and photophobia. Some patients may be intermittently asymptomatic or mildly symptomatic. Keratitis may be severe, but rarely, if ever, leads to corneal ulceration (Table 7).

Intestinal and Biliary Tract Microsporidiosis in HIV Infection

Chronic diarrhea and wasting are common and frequently serious complications of HIV infection (9, 59, 88, 198). *Enterocytozoon bieneusi* is estimated to be one of the most important intestinal pathogens in severely immunodeficient HIV-infected patients, present in 7 to 50% of those with otherwise unex-

plained chronic diarrhea (Table 4). *S. intestinalis* is a less commonly recognized cause of chronic diarrhea (Table 6).

Enterocytozoon bieneusi. *E. bieneusi* microsporidiosis is most common in patients with severe cellular immunodeficiency, i.e., when CD4 cell counts drop below 50 to 100/ml^3. When microsporidiosis is diagnosed, the majority of the patients have had other opportunistic infections. The main symptoms are chronic nonbloody diarrhea without fever, anorexia, weight loss of about 2 kg/month, and bloating. Some patients experience intermittent diarrhea, and a few excrete microsporidial spores without having diarrhea (2, 22, 138, 147, 166, 233). The stool is watery or soft, and the number of bowel movements is usually about three to seven per day, rarely, up to 20 per day. Some authors have noted that diarrhea seems to be worsened by most foods and tends to be more frequent in the mornings (2, 147, 149). About half of the patients report abdominal pain, and some have nausea and vomiting. Physical findings, if present, are nonspecific. Laboratory evidence for intestinal malabsorption is common but also nonspecific. Long-term observations of up to 3 years have indicated that *E. bieneusi* itself is not immediately life-threatening (205, 236), but diarrhea is debilitating, and weight loss may be massive, leading to cachexia, which is a significant cause or cofactor in the deaths of many patients (77, 138, 233). Up to one-third of patients with intestinal microsporidiosis have dual or multiple coinfection with other intestinal pathogens, which may occur simultaneously or sequentially (228, 229, 233, 237, 238).

Clinically symptomatic extraintestinal dissemination of *E. bieneusi* along mucosal surfaces seems uncommon, but recently it has also been detected in the biliary tree of patients with cholangitis and, rarely, in the gallbladder of patients with acalculous cholecystitis (10, 131, 163, 164). These observations suggest that microsporidiosis might be an important cause of the so-called AIDS cholangiopathy which also has been associated with cytomegalovirus and cryptosporidial infection (20, 23, 46, 126, 130). In most patients with recognized biliary *E. bieneusi* infection, chronic diarrhea is also present and right upper quadrant abdominal pain is common. Imaging procedures (abdominal sonography and computed tomography, endoscopic ultrasonography, and endoscopic retrograde cholangiopancreatography) often reveal dilatation of both intrahepatic and common bile ducts, irregularities of the bile duct wall, and gallbladder abnormalities such as wall thickening, distension, and the presence of sludge. Papillary stenosis may also be present, but clinical jaundice is rarely evident. Laboratory values for serum alkaline phosphatase are usually elevated (most often two to three times the upper limit of normal); total bilirubin concentrations and aspartate and alanine aminotransferase levels in serum are generally normal (164).

S. intestinalis. Clinical manifestations of chronic diarrhea and weight loss are similar to *Enterocytozoon bieneusi* infection. *S. intestinalis* may also spread into the biliary tract and into the gallbladder, causing cholangitis and cholecystitis (150). In contrast to *Enterocytozoon bieneusi*, systemic dissemination to kidneys and other sites without a luminal connection to the intestine may occur, but intestinal symptoms appear to predominate (2, 150, 231, 239).

Other intestinal microsporidia. A *Nosema*-like microsporidian was identified in the stool specimen of one patient with AIDS who had suffered from diarrhea, nausea, and anorexia for several months. Because the microsporidial organisms found in the specimen were enclosed within striated muscle cells, the authors concluded that they were probably ingested in food, representing an incidental finding rather than a true infection (129). *Nosema* infections have not been documented in HIV-infected persons (29, 42).

Microsporidial Hepatitis and Peritonitis in HIV Infection

Hepatitis due to *Encephalitozoon* sp., classified as *Encephalitozoon cuniculi* on the basis of ultrastructure, has been observed in a 35-year-old HIV-infected patient with Kaposi's sarcoma and a CD4 cell count of 48/ml^3. His illness began with fatigue, intermittent diarrhea, and weight loss. Four months later, he developed jaundice, fever, and severe diarrhea, had a rapid worsening of hepatocellular necrosis, and died (207).

Peritonitis attributed to *Encephalitozoon cuniculi* has been described in a 45-year-old man with 57 CD4 cells per ml^3. He had lost 13 kg of body weight during the previous year and was hospitalized because of *Pneumocystis carinii* pneumonia. Two days after therapy with trimethoprim-sulfamethoxazole was initiated, he developed acute renal failure. In addition, a large palpable tumorlike mass was found in the abdomen. Broad-spectrum antibiotics were given because of persistent fever. He developed a subacute ileus and died 40 days after admission. At limited autopsy, the mass, corresponding to the omentum magnum, showed a nongranulomatous inflammation that contained microsporidia consistent in ultrastructure with *Encephalitozoon cuniculi* (253).

Enterocytozoon bieneusi and *S. intestinalis* have also been identified in nonparenchymal liver cells of patients with microsporidian-associated cholangitis and diarrhea (150, 164). The patients, however, did not show signs or symptoms of hepatitis.

Systemic Microsporidiosis in HIV Infection

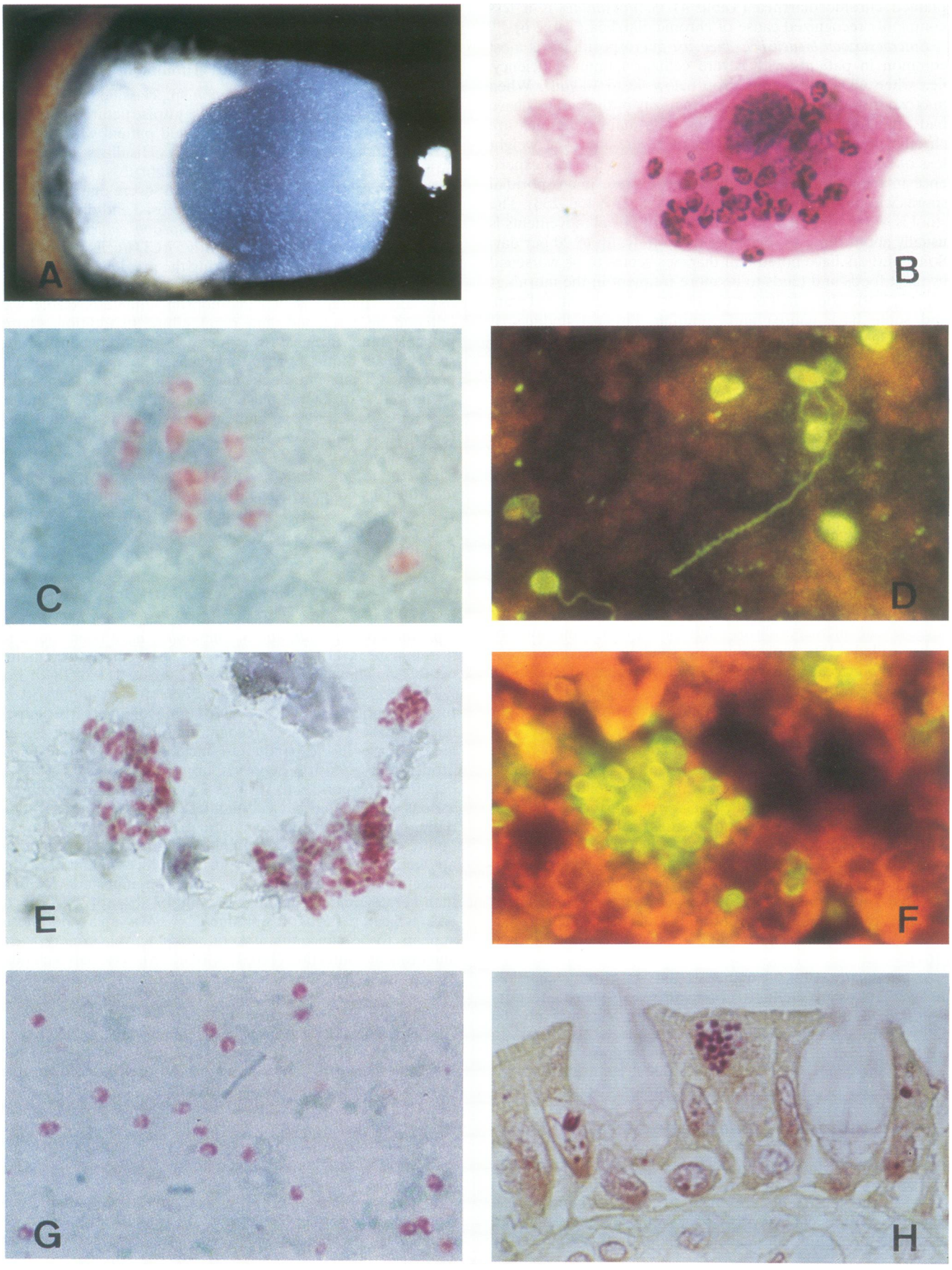
Three microsporidial species, *Encephalitozoon hellem*, *Encephalitozoon cuniculi*, and *S. intestinalis*, have been found to disseminate in HIV-infected persons, particularly in patients with CD4 cell counts below 50/ml^3 (2, 62, 150, 180, 184, 235).

***Encephalitozoon* spp.** *Encephalitozoon* sp. was initially identified in patients with AIDS and keratoconjunctivitis. Subsequently, the spectrum of recognized *Encephalitozoon*-associated disease has expanded to include keratoconjunctivitis, bronchiolitis, sinusitis, nephritis, ureteritis, cystitis, prostatitis, hepatitis, and peritonitis (Tables 2 and 5). Preliminary observations suggest that clinical manifestations may vary substantially, ranging from an asymptomatic carrier state to organ failure (180, 184, 235). In patients who present with symptomatic keratoconjunctival microsporidiosis, dissemination of the parasite may be common, but clinical manifestations other than keratoconjunctivitis may be mild or absent (25, 235).

A typical pattern of systemic *E. hellem* microsporidiosis appears to be concomitant keratoconjunctival, urinary tract, and bronchial infection (180, 184, 185, 235). Associated clinical presentations may include keratoconjunctival inflammation, cystitis, nephritis, renal failure, bronchitis, pneumonia, and, possibly, progressive respiratory failure.

Severe multiorgan system involvement with *E. hellem* and autopsy findings were described in 1992 in a 30-year-old Hispanic man with AIDS in whom the earliest recognizable signs of encephalitozoonosis were flank pain, dysuria, and gross hematuria. Cystoscopy showed hemorrhagic cystitis, and biopsy revealed microsporidia which subsequently also were repeatedly found in urine specimens. The patient was treated with various antimicrobial agents, but respiratory and renal failure were progressive and he died (180).

In 1993, another 27-year-old man with AIDS and conjunctival and urinary tract infection with *E. hellem* who developed a persistent nonproductive cough, wheezing, pleuritic chest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pain, and fever was reported. Chest roentgenogram showed left lower lobe interstitial infiltrates. *E. hellem* spores were detected in sputum and bronchoalveolar fluid (Fig. 17C and D), and microsporidial bronchiolitis was confirmed with transbronchial biopsy (Fig. 18). The patient died after a 2-month course of persistent fever, increasing shortness of breath, and worsening pleuritic chest pain (184).

In yet another case of systemic encephalitozoonosis, culture of the parasites, antigenic analysis, and results of molecular analysis suggested that the infecting species was *E. cuniculi* (62). The patient experienced fever, night sweats, intractable cough, sinus pain and congestion, dry eyes, and blurred vision. Punctate keratopathy, interstitial pneumonia, sinusitis, and progressive renal insufficiency were diagnosed, and microsporidia were detected in lung biopsy tissue, conjunctival smears, sputum, urine, and a smear of an ulceration on the tongue. Treatment with albendazole was effective.

S. intestinalis. Infection with *S. intestinalis* appears to be mainly confined to the small intestine and biliary tract as described above, but dissemination into kidneys and bronchial epithelium has been found. Clinical manifestations of systemic infection, however, have yet to be defined, but interstitial nephritis, possibly leading to renal failure, may ensue (Table 6) (150).

Urinary Tract Microsporidiosis in HIV Infection

Microsporidial infection confined to the urinary tract has not been reported, but evaluation of predominant genitourinary signs and symptoms in HIV-infected patients may include consideration of microsporidial infection. Only single case reports of urinary tract infection due to *Encephalitozoon hellem* and *S. intestinalis* have been described, and little is known about the clinical presentation and consequences of the presence of microsporidia in the renal and urinary systems. Preliminary observations indicate that infection may be latent with and without asymptomatic microhematuria (235), may cause cystitis and interstitial nephritis associated with dysuria and gross hematuria, and possibly may lead to progressive renal failure (97, 180). Microsporidia have also been detected in the prostate of a patient with systemic encephalitozoonosis (186).

Respiratory Tract Microsporidiosis in HIV Infection

Prevalence data on pulmonary microsporidial colonization are not available, but microsporidian-associated pulmonary illness has, infrequently, been reported (38, 62, 76, 78, 90, 113, 180, 182, 184, 185, 234, 235). In most patients with respiratory tract microsporidial infection, intestinal and/or systemic microsporidiosis was also present. Nevertheless, single cases of patients in whom sinonasal *Enterocytozoon bieneusi* (76, 90), *Encephalitozoon* spp. (38, 76, 90, 113), and *S. intestinalis*-associated (79, 182) infections were a predominant manifestation of systemic microsporidiosis have been reported. The patients experienced sinusitis and/or nasal polyposis, causing nasal obstruction and persistent mucopurulent nasal discharge.

These microsporidia have also been identified in the lower respiratory tract, associated rarely with bronchiolitis, pneumonia, and progressive respiratory failure. One case of pulmonary involvement with *Enterocytozoon bieneusi* has been reported in a patient with chronic diarrhea who developed a persistent cough with scant, nonpurulent sputum, dyspnea, and wheezing. Chest roentgenogram showed minimal interstitial infiltrates and a small pleural effusion. *Enterocytozoon bieneusi* spores were detected in bronchoalveolar lavage fluid, transbronchial biopsy specimens of the posterior segment of the left lower lobe, stool, and ileal biopsy specimens (234). Two well-documented patients with symptomatic pulmonary *Encephalitozoon* infection are discussed above (62, 184). Also, asymptomatic pulmonary colonization with *Encephalitozoon hellem* has been described in a patient with AIDS without microsporidian-associated signs or symptoms in whom repeated examination of sputa, urine, and conjunctival smears revealed *Encephalitozoon hellem* spores (235). *S. intestinalis* has been detected in bronchial epithelial cells at autopsy, but it is not known whether this species causes pulmonary illness (150, 231).

DIAGNOSIS

Diagnosis of microsporidial infection is dependent on morphological demonstration of the organisms themselves by light or electron microscopic examination (Tables 8 and 9). Serological tests to detect antibodies to *Encephalitozoon* spp. have been developed, but available results suggest that these tests may not be feasible for diagnosis of human microsporidiosis, particularly in patients with immunodeficiency (41, 69). *Encephalitozoon* sp. and *Nosema* sp. have been isolated by using cell culture systems, but these tests are fastidious and costly, and the most common human species, *Enterocytozoon bieneusi*, has not been continuously propagated (222). Molecular probes are being developed but are currently not available outside of research laboratories (64, 89, 94, 160, 226, 227, 254–256).

Detection of microsporidial parasites has often been based on electron microscopic examination of tissue specimens because of the organisms' small size; staining properties that sometimes hamper visualization of the spores and developing stages, using routine staining techniques; and the unfamiliarity of pathologists and parasitologists with the histopathologic

FIG. 17. (A) Slit lamp demonstration of punctate epithelial keratopathy in a patient with AIDS and keratoconjunctivitis due to *Encephalitozoon hellem* (photograph courtesy of Michael Diesenhouse). (B) Conjunctival smear from a patient with AIDS and keratoconjunctivitis due to *E. hellem* (same patient as panel A). Numerous microsporidia are present within the cytoplasm of an infected epithelial cell. Gram stain was used. Magnification, ×1,000. (C) Sputum smear from a patient with AIDS and disseminated *E. hellem* infection stained with Weber's chromotrope stain, clearly demonstrating the pink-stained spores of *E. hellem*. Chromotrope staining technique was used. Magnification, ×1,000. From reference 184, with permission of the publisher. (D) Fluorescent-antibody staining of sputum, using antibody to *E. hellem* from a patient with disseminated microsporidiosis and symptomatic bronchial infection. Immunofluorescence technique and fluorescein-tagged antibodies to *E. hellem* were used. Magnification, ×1,000. From reference 184, with permission of the publisher. (E) Cytology of a urine specimen centrifuged at 1,500 × *g* from a patient with AIDS and disseminated *E. hellem* infection. The smear is stained with Weber's chromotrope stain, showing the intracellular pink-stained spores of *E. hellem*. Chromotrope staining technique was used. Magnification, ×630. (F) Tissue section obtained following autopsy of a patient with disseminated microsporidiosis. Microsporidia in the renal tubules are demonstrated by using fluorescein-tagged antibodies to *E. hellem*. Immunofluorescence technique and fluorescein-tagged antibodies to *E. hellem* were used. Magnification, ×1,000. From reference 184, with permission of the publisher. (G) Smear of a formalin-fixed, unconcentrated stool specimen of a patient with AIDS and chronic diarrhea, showing pinkish-red-stained spores of *Enterocytozoon bieneusi*. Chromotrope staining technique was used. Magnification, ×1,000. (H) Terminal ileal tissue obtained by ileocolonoscopy in a patient with AIDS and chronic diarrhea. The supranuclear location of the gram-positive spores of *E. bieneusi* is a characteristic finding. Brown-Brenn stain was used. Magnification, ×1,000.

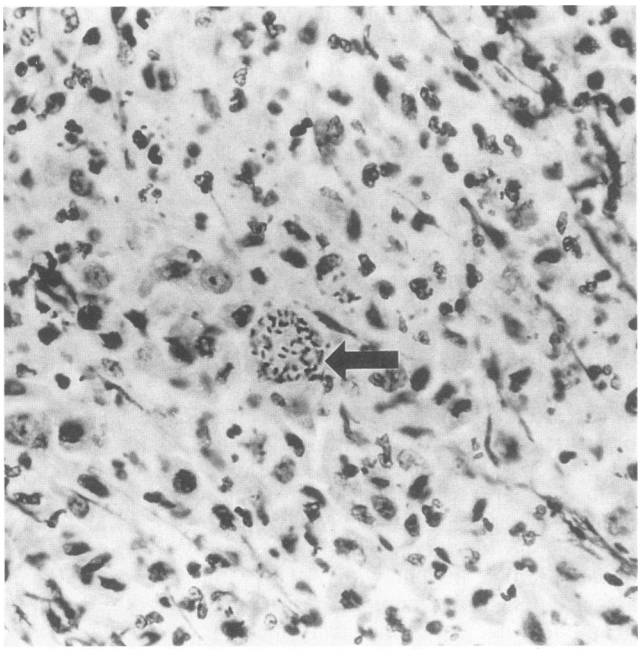

FIG. 18. Tissue section of a transbronchial biopsy in a patient with AIDS, bronchiolitis, and disseminated *Encephalitozoon hellem* infection. A group of microsporidial spores (arrow) are present in an inflamed bronchiole. Tissue Gram stain (Brown & Hopps) was used. Magnification, ×400.

appearance of this infection. The small spores, the stages by which microsporidia usually are identified, range in size from 1 to 2.5 μm in most species found in humans.

Although electron microscopy is often necessary to establish definitive species identification on the basis of ultrastructural features, the initial detection of microsporidia in tissue specimens can be accomplished with light microscopy alone. Moreover, the cytologic evaluation of more readily obtainable specimens such as stool, duodenal aspirates, urine, sputum, nasal discharge, bronchoalveolar lavage fluid, and conjunctival smears by light microscopic examination is now well described and is becoming routine practice (169, 181, 183, 184, 187, 220, 232, 234, 235).

Effective morphological demonstration of microsporidia by light microscopy is best accomplished with staining methods that produce distinct contrast between staining of the very small microsporidial spores and other cellular contents or background debris. Such staining properties are particularly useful for the examination of stool specimens and other bodily fluids heavily laden with bacteria and other debris, because no concentration method that allows consistent separation of microsporidia from other particles is available for routine use (232). A coprodiagnostic technique based on chromotrope staining, recently developed by Weber and colleagues, has aided in the diagnosis of intestinal microsporidiosis (232), and the technique has also been successfully used to identify microsporidial spores in duodenal aspirate, urine, bronchoalveolar lavage fluid, sputum, and conjunctival smears (183, 184, 232, 234, 235). Independent of the type of specimen or staining technique, light microscopic identification of spores requires adequate illumination and magnification, i.e., ×630 or ×1,000 (oil immersion).

Light Microscopic Examination of Stool Specimens

Chromotrope staining. The chromotrope-based staining technique for light microscopic examination of stool specimens (Fig. 17G) includes steps similar to those used in the trichrome staining procedure of Wheatley (245), which is routinely used for parasitologic examination of stool specimens in many laboratories in the United States. The chromotrope concentration of the new staining solution, however, is 10-fold higher, and the exposure time of the smear to the staining solution is prolonged (232). Ten-microliter aliquots of a suspension of unconcentrated stool fixed in 10% formalin are very thinly spread on a slide. Smears are fixed in methanol for 5 min and stained for 90 min with the chromotrope-based stain. After staining, slides are rinsed in acid alcohol for 10 s and then rinsed briefly in 95% alcohol. Smears are then successively dehydrated in 95% alcohol for 5 min, 100% alcohol for 10 min, and Hemo-De (a xylene substitute; Fisher Scientific, Pittsburgh, Pa.) for 10 min. The slides are scanned at ×1,000 magnification (oil immersion).

The chromotrope-based staining solution is prepared by mixing 6.0 g of chromotrope 2R, 0.15 g of fast green, and 0.7 g of phosphotungstic acid. After these ingredients have stood for 30 min in 3 ml of glacial acetic acid, they are mixed with 100 ml of distilled water. Acid alcohol for rinsing is prepared with 4.5 ml of acetic acid and 995.5 ml of 90% ethyl alcohol (232).

Spores of *Enterocytozoon bieneusi* measure approximately 0.9 by 1.5 μm, are ovoid and refractile, and have a specific appearance when stained with this technique. The spore wall stains bright pinkish-red; some spores appear transparent, and others show a distinct pinkish-red-stained, beltlike stripe that girds the spores diagonally or equatorially (232). Most background debris in stool specimens counterstains faint green. Some other fecal elements, such as yeasts and some bacteria, may also stain reddish, but they are distinguished from microsporidial spores by their size, shape, and staining pattern. Spores of *S. intestinalis* and *Encephalitozoon* spp. measuring 1.0 to 1.5 by 2.5 to 3.0 μm appear consistently and significantly bigger than those of *Enterocytozoon bieneusi* and show staining patterns similar to that of *Enterocytozoon bieneusi* (235, 239).

Ryan and colleagues proposed a modification of Weber's chromotrope staining solution in which aniline blue (0.5 g for 100 ml of staining solution) has been substituted for fast green (which stains the background) and there has been a reduction in the level of phosphotungstic acid (0.25 g). The concentration of chromotrope (which stains the microsporidial spores) was identical to that originally described (172). Kokoskin and colleagues evaluated changes in temperature of the standard chromotrope staining solution and staining time. Their results suggest that staining at a temperature of 50°C for 10 min improved the detection of microsporidia. The background was clearer, and spores stained more intensely (107).

Parasitologists may not be accustomed to scanning for structures of the size of microsporidia by light microscopy, and the spores are indeed so small that they may be overlooked when attention is not directed to their detection, or when microscopic examination technique is inappropriate. Nevertheless, when positive control slides are available, microscopists are able to reliably use light microscopic stool examination procedures for diagnosis of microsporidiosis after adequate training, and the technique easily fits into a clinical laboratory's workflow. Epidemiologic evaluation of the method by different investigators has shown a sensitivity and specificity which are at least equal to results of light and electron microscopic examination of tissue sections (63, 174, 179, 205, 221, 232, 237).

TABLE 8. Microscopical detection of microsporidia

Specimen	Detection of parasite (reference[s])[a]		
	Enterocytozoon bieneusi	*Encephalitozoon* species	*Septata intestinalis*
Tissue			
Duodenum, jejunum	++ (149)	−	++ (150)
Terminal ileum	++ (154)	−	−
Colon	+ (166)	−	+ (150)
Biliary tract, gallbladder	+ (164)	−	+ (150)
Liver	+[b] (164)	+ (207)	+[c] (150)
Pancreatic duct	+ (27)	−	−
Peritoneum	−	+ (253)	−
Bronchial epithelium	+ (234)	+ (180)	+ (150)
Trachea	+ (148)	+ (180)	−
Sinus, nasal epithelium, nasal polyps	+ (76)	+ (113)	+ (79, 182)
Cornea, conjunctiva	−	++ (48)	−
Kidney	−	+ (180)	+ (150)
Ureter	−	+ (180)	−
Urinary bladder	−	+ (180)	−
Prostate	−	+ (186)	−
Bodily fluids or mucosal smears			
Duodenal aspirate	++ (155, 232)	−	+ (239)
Bile	+ (164)	−	−
Bronchoalveolar lavage fluid	+ (234)	++ (184, 185, 187)	−
Sputum	−	++ (184, 185, 187, 235)	+ (79, 182)
Nasal secretion (swab), nasal washing	+ (76)	+ (38)	+ (79, 182)
Urine	−	++ (223)	+ (150)
Stool	++ (219, 220, 232)	−	++ (79, 150, 239)
Cerebrospinal fluid	−	+ (127)	−
Conjunctival smear	−	++ (183)	−
Blood	−	−	−

[a] −, not reported; +, single case report(s) of detection; ++, consistently reliable specimens for detection of microsporidia.
[b] *Enterocytozoon bieneusi* was present in ductal biliary cells, but not in parenchymal liver cells, in the liver biopsy of one patient.
[c] *S. intestinalis* was detected in nonparenchymal liver cells in the autopsy specimens of one patient.

Stool concentration methods. None of the procedures that are routinely used to concentrate ova and parasites results in a concentration of microsporidial spores in stool specimens (104, 232). The formalin-ethyl acetate concentration procedure or different flotation methods produce significant removal of fecal debris, and smears prepared from these concentrates might appear easier to read by light microscopic examination compared with smears from unconcentrated specimens. Such concentration techniques, however, lead to a substantial loss of microsporidial spores and false-negative results (232). New concentration techniques, including modifications in centrifugation times and speeds, have been proposed or are being investigated, but epidemiologic evaluation has to confirm whether such techniques may lead to adequate or improved recovery of spores (218).

Chemofluorescent agents. Chemofluorescent optical brightening agents (Calcofluor White 2MR; American Cyanamid Corp., Princeton, N.J.; Fungi-Fluor Kit, Polysciences Inc., Warrington, Pa.; Uvitex 2B, Ciba Geigy, Basel, Switzerland), which require examination with a fluorescence microscope, also stain microsporidial spores (99, 220). The chitinous wall of the microsporidial spores is brightened, but staining is not specific. Fungi, of which small species may be present in fecal material, and other fecal elements may also be brightened, and brightened microsporidial spores do not show a specific morphology (220, 232). Smears containing high numbers of spores are indeed easy to read by experienced microscopists using chemofluorescent agents. Nevertheless, we do not use these techniques for routine examination of stool specimens because distinct differentiation of spores from other fecal elements may be difficult, which may lead to false-positive results.

Giemsa staining of stool specimens. Giemsa staining results in a light-blue staining of microsporidia, and sometimes a characteristic darkly stained nucleus may be visualized (219). The very small, blue-stained microsporidial spores, however, are difficult to identify in smears of stool specimens and to differentiate from other fecal elements, which all are also blue stained.

Cytologic Diagnosis

Microsporidial spores have been detected in sediments of duodenal aspirate, bile or biliary aspirates (obtained with endoscopic papillary cannulation, transhepatic catheterization, and during surgical cholecystectomy [164]), urine, bronchoalveolar lavage fluid, and cerebrospinal fluid and in smears of conjunctival swabs, sputum, and nasal discharge (25, 62, 127, 183–185, 220, 223, 224, 232, 234, 235). Because microsporidia are so small, high-speed centrifugation (at least 1,500 × *g*) of bodily fluids, e.g., urine or duodenal aspirate, may be necessary to concentrate organisms in sediments. For cytologic examination of bodily fluids that do not contain substantial background debris, bacteria, or fungi, staining with Gram stain (microsporidial spores are often gram variable and stain partially gram positive or dark reddish), Giemsa stain, and chemofluorescent agents may be useful. Portions of the spore structure of some microsporidial species may stain acid fast but we have not been successful in using Ziehl-Neelson stain or modified acid-fast

TABLE 9. Diagnostic techniques for detection of microsporidia[a]

Technique	Recommended for routine use	Remarks (reference[s])
LM		
Stool specimens		
Chromotrope stain	++	Reliable, readily available (172, 232)
Giemsa stain	−	Used by some investigators [219] but difficult to differentiate microsporidial spores from other fecal elements
Chemofluorescent agents	+	Calcofluor, Uvitex 2B [220]; sensitive but not specific; other fecal elements are also stained; needs further validation
IF technique	−	Anti-*Enterocytozoon* antibodies not available; Cross-reacting IF tests reported (83, 244, 257)
Other bodily fluids		
Chromotrope stain	++	Reliable, readily available (172, 232, 235)
Giemsa, Gram stain	+	Useful for examination of urine, conjunctival swab, BAL, CSF, duodenal aspirate (25, 29)
Chemofluorescent agents	+	Sensitive, not specific [220]
IF technique	++	Fluorescein-tagged anti-*Encephalitozoon* antibodies useful but not widely available (83, 180, 223, 244, 257); anti-*Enterocytozoon* antibodies not available
Touch preparation		
Giemsa	+	Sensitivity not evaluated; preferred by some authors (169)
Paraffin-embedded tissue sections		
Hematoxylin & eosin stain	+	Sensitivity with low parasite load uncertain; usefulness debated
PAS stain	+	Not useful for detection of *Enterocytozoon* spp.; useful for detection of *Nosema* spp. and other microsporidia (125)
Modified Gram stains (Brown Brenn, Brown-Hopps)	++	Sensitive; not standardized (232)
Giemsa stain	−	Not sensitive when low parasite load
Warthin-Starry stain	+	Sensitive, specific in one study (78); not standardized; promising
Chromotrope stain	++	Appeared sensitive, specific (85) but not evaluated in epidemiologic studies
IF technique	++	Fluorescein-tagged anti-*Encephalitozoon* antibodies useful but not widely available (1, 180, 223); anti-*Enterocytozoon* antibodies not available
Plastic-embedded tissue sections		
Toluidine stain	+	(149)
Methylene blue-azure II-fuchsin stain	+	(149)
EM		
Bodily fluid	+	Specific but sensitivity unknown; useful for identification of species (155, 232, 234, 238, 239)
Tissue sections	++	Gold standard for confirmation; but sensitivity may be lower than that of techniques for detection of spores in stool and urine specimens; identification of species
Serological antibody detection		
Encephalitozoon serology	−	Sensitivity and specificity unknown; controversial interpretation of results
Enterocytozoon serology	−	Not available
Culture of parasites	−	*Encephalitozoon* and *Nosema* spp. can be isolated in cell culture systems (41, 68, 96, 189, 193, 195, 223); *Enterocytozoon* spp. not grown as yet (222)
PCR	−	Not available outside research laboratories; sequences of small rRNA gene of some human microsporidia published (64, 89, 90, 94, 160, 226, 254–256)

[a] LM, light microscopic examination; EM, electron microscopic examination; IF, immunofluorescence detection procedure; BAL, bronchoalveolar lavage fluid; CSF, cerebrospinal fluid; PAS, Periodic acid-Schiff. −, not available or not recommended as a diagnostic technique for routine use; +, reported; ++, techniques used by the authors.

staining techniques to detect *Enterocytozoon bieneusi* in stool specimens or *Encephalitozoon* spp. in urine. Diagnostic confidence may be attained by using the chromotrope-based staining technique, which has been favorably applied to identify microsporidial spores in duodenal aspirate, urine, bronchoalveolar lavage fluid, sputum, and conjunctival smears (Fig. 17C and E) (183, 184, 232, 234, 235).

Because microsporidial spores may be shed periodically, as is seen in mammalian encephalitozoonosis (21, 55), repeat examination of single urine specimens or a 24-h urine collection may be necessary for detection of *Encephalitozoon* spores.

Histologic Examination

For examination of tissue sections, various staining techniques have been tried. Only highly experienced pathologists

have reliably and consistently identified microsporidia in tissue sections by using routine techniques (147, 149, 157). Even in experienced hands, microsporidia are easily missed in hematoxylin-and-eosin-stained sections, particularly when organisms are focally distributed or present in low numbers (78, 166). Giemsa staining of touch preparations of small-intestinal tissue may be useful, but this technique has never been evaluated in large clinical or epidemiologic studies (169, 196). Ultrathin plastic sections stained with methylene blue-azure II-basic fuchsin or with toluidine blue may facilitate detection, but these techniques are not routinely used (149). In our experience, tissue Gram stains such as Brown-Brenn or Brown-Hopps have proven to be the most useful for the rapid and reliable identification of HIV-associated microsporidia in routine paraffin-embedded tissue sections (Fig. 10 and 17E) (29, 232, 236). *Enterocytozoon bieneusi* spores are often gram variable, but they are readily identified because of the contrasting dark blue or reddish staining against a faint brown-yellow background. Nonspore stages, however, are not visualized by this technique. Others prefer a silver stain (Warthin-Starry stain) for visualizing both spore and intermediate stages for both *Enterocytozoon bieneusi* and *S. intestinalis* (78) or the chromotrope-based staining technique described by Giang and colleagues (85).

Because microsporidia are very small, may occur in the absence of noticeable inflammatory tissue reaction, and may be focally distributed, attentive examination and adequate light microscopic magnification, i.e., ×630 or ×1,000 (oil immersion), are required for identification of microsporidia independent of the staining technique used (232).

Examination of biopsy and autopsy specimens from various anatomic sites has resulted in diagnoses of human microsporidiosis. Examples include cornea, conjunctiva, skeletal muscle, small and large intestine, liver, gallbladder, bile duct, pancreatic duct, omentum, kidney, ureter, bladder, prostate, and trachea or bronchi.

Because *Enterocytozoon bieneusi* is most reliably detected in the enterocytes at the tips of villi by both light microscopy and electron microscopy, histologic diagnosis of intestinal microsporidiosis is facilitated by orientation of intestinal biopsies prior to fixation. Endoscopic biopsies are folded cut surface to cut surface by the two jaws of the biopsy forceps. The two halves of intestinal biopsies can be teased apart with a blunt probe, and the cut surface can be affixed to paper by contact. When the biopsy is removed from fixative, the paper base can be removed or used for orienting the biopsy in embedding medium, so that sections, taken perpendicular to the cut edge, give crypt-to-tip profiles of villi.

Round to oval *Enterocytozoon bieneusi* spores are characteristically located in the cytoplasm of enterocytes between the microvillous border and the nucleus. They must be differentiated from enteroendocrine cell granules, which appear similar to spores but are located between the enterocyte nucleus and the basal lamina. Plasmodia often stain lighter than surrounding host cytoplasm and are characterized by a cleft (the electron-lucent inclusion), which is present in all developmental stages of *Enterocytozoon bieneusi* (149). Nonspore stages can be inferred, even when not visualized, if they indent the superior pole of the enterocyte nucleus, toward the microvillus border.

Since microsporidia can only penetrate and infest host enterocytes after they emerge from intestinal crypts, enterocytes are more likely to be parasitized the longer they have been exposed to luminal spores. Consequently, enterocytes are most heavily parasitized at villus tips, where they are most likely to contain mature spore forms. Within crypts, granules of Paneth cells should not be mistaken for spores of *Enterocytozoon bieneusi*, which are not found in crypts, due to the absence of enterocyte penetration by spore polar tubules in this location.

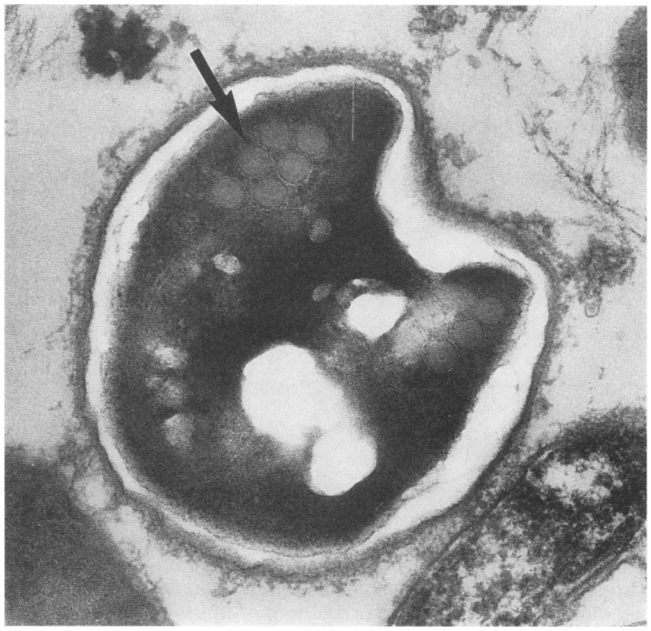

FIG. 19. Transmission electron micrograph of a microsporidial spore from a stool specimen of an HIV-infected patient with chronic diarrhea. The polar tubes (arrow) lie in two rows characteristic of *Enterocytozoon bieneusi*. Magnification, ×51,000.

Spores of *S. intestinalis* are segregated from the enterocyte cytoplasm in vacuoles and are more easily recognized than spores of *Enterocytozoon bieneusi*. Unlike *Enterocytozoon bieneusi*, *S. intestinalis* can also be seen in the intestinal lamina propria below the basal lamina, clustered in vacuoles.

Electron Microscopy

Until recently, electron microscopic examination was considered essential to the identification of these organisms, but improved light microscopic examination procedures and immunologic and biochemical as well as molecular techniques being developed may facilitate the diagnostic workup. Nevertheless, microsporidial ultrastructure is unique and pathognomonic for the phylum, and, with rare exceptions, ultrastructural features also can distinguish between most genera of microsporidia (31, 36, 38). Accurate taxonomic classification of microsporidia is essential because different genera and species exhibit different biologic and epidemiologic characteristics that influence approaches to prevention and treatment.

Examination of bodily fluids. Following the description by van Gool and colleagues of the recovery of microsporidial spores from fecal specimens (219) and by Visvesvara and colleagues of the diagnosis of disseminated microsporidiosis by examination of urinary sediment (223), detection and species identification of microsporidia from stool and urine specimens using electron microscopy have repeatedly been reported (Fig. 19 and 20) (150, 155, 174, 175, 232, 239). A noninvasive coprodiagnostic approach, including ultrastructural differentiation of spores of *Enterocytozoon bieneusi* and *S. intestinalis* in stool specimens, may be important for patient care, because infection due to *S. intestinalis* can be successfully treated with albendazole (2, 239). Spore size and ultrastructure, particularly

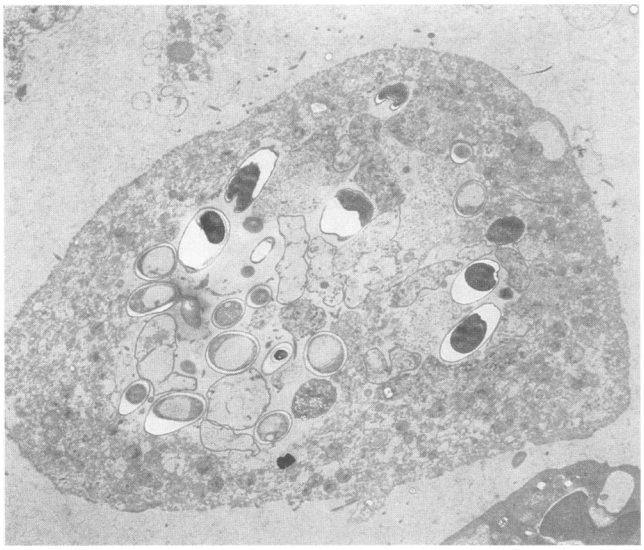

FIG. 20. Transmission electron micrograph of urine sediment of a patient with AIDS and disseminated *Encephalitozoon hellem* infection showing intracellular microsporidial spores. Magnification, ×2,924.

in regard to the configuration of the coiled tubules, differ between *Enterocytozoon bieneusi* (tubules arranged in two rows, Fig. 19) and *S. intestinalis* (tubules arranged in one row) (239). However, spore morphology of *Septata* and *Encephalitozoon* spp. (which has never been identified in a human intestine and has not been associated with diarrhea in HIV-infected patients so far) is almost identical, and differentiation of these species on the basis of spore ultrastructure may not be achievable by examination of bodily fluids (239).

Examination of tissue sections. Transmission electron microscopic examination of tissues sections will remain a valuable adjunct to diagnosis and clinical management and is often indicated for diagnostic and species confirmation (Fig. 5 to 9) (25). Major ultrastructural features for species identification are the characteristics of developmental stages of the parasite and the host-parasite interface, which are observed only in tissue specimens, as well as the nuclear configuration and spore morphology. In contrast, electron microscopic examination of stool or urine specimens may reveal only spore ultrastructure.

Immunofluorescence Detection Procedures

Fluorescein-tagged polyclonal antibodies have been used by some investigators for histologic and cytologic detection of microsporidia in human specimens (Fig. 17D and F) and to visualize different microsporidial developmental stages in cell cultures (1, 83, 144, 180, 183, 184, 223, 235, 244, 257). Species-specific polyclonal antibodies have enabled investigators to distinguish between morphologically identical species such as *Encephalitozoon hellem* and *Encephalitozoon cuniculi* (180, 183, 184, 223). Taking advantage of cross-reactivity between *Encephalitozoon* antisera and *Enterocytozoon bieneusi* antigens, polyclonal antibodies have also been used to demonstrate spores of *Enterocytozoon bieneusi* in stool and intestinal biopsy tissue (1, 83, 244, 257). Monoclonal antibodies for use in immunofluorescence staining are under development (1).

Serology

Serologic assays (including carbon immunoassay, indirect immunofluorescence test, enzyme-linked immunosorbent assay [ELISA], and Western blot immunodetection) have been useful in detecting antibodies to *Encephalitozoon cuniculi* in several species of animals (45, 217), but reliable serologic tests for diagnosis of human microsporidiosis are lacking. This unavailability is due, in part, to the fact that *Enterocytozoon bieneusi* has not been continuously propagated in cell culture or laboratory animals. Furthermore, development, use, and interpretation of serologic assays to detect antibodies to any parasite is notably challenging in the immunocompromised host. Recent studies have demonstrated a high degree of variability in ELISA- and Western blot-measured human antibody responses to a variety of microsporidial antigens of both human and insect origin, suggesting that serologic tests may not become feasible for diagnosis of microsporidiosis, specifically in patients with AIDS (69, 244).

Seroepidemiologic studies in different human populations have suggested that antibodies that react with *Encephalitozoon cuniculi* may be quite prevalent in some groups of patients (11, 12, 41, 68, 69, 95, 98, 197, 246). In patients with a history of tropical protozoal infections other than microsporidia, and in persons living in or having travelled to tropical countries, seroprevalences ranging from 0 to 42% have been reported. The sensitivity and specificity of such tests in humans, however, are unknown, and it is uncertain whether detection of antibodies to *Encephalitozoon cuniculi* represents true infection, cross-reactivity to other microsporidial species, or nonspecific reactions (25, 29, 41). The antigens used in these surveys were derived from cell cultures of murine strains of *Encephalitozoon cuniculi*, but recent findings indicate that human encephalitozoonosis may be mostly caused by the species *Encephalitozoon hellem* (68). To date, in only one human case (the 2-year-old Colombian boy living in Sweden) was detection of antibodies to *Encephalitozoon cuniculi* in serum accomplished prior to the identification of organisms in urine specimens (11). Whether the result of the serologic test reflected cross-reactivity between different species of *Encephalitozoon* or whether the child indeed was infected by *Encephalitozoon cuniculi* is unknown.

Cell Culture

Culture techniques for routine diagnosis of human microsporidiosis are not feasible because microsporidia do not grow on axenic medium. Propagation of some species can be achieved in cell culture using several different cell lines (41, 68, 96, 97, 189, 193, 195, 223). Three species infecting humans have successfully been isolated from human specimens: *Encephalitozoon hellem* has been grown from conjunctival and corneal tissue and scrapings, as well as urine, sputum, and bronchoalveolar lavage fluid (Fig. 3 and 4) (68, 180, 184, 223, 224); *N. corneum*, from corneal tissue (193); and *S. intestinalis*, from stool specimens and urine (96). Various attempts to culture *Enterocytozoon bieneusi* have not achieved long-term cultures, but short-term propagation has been described (222).

Approach to Diagnosis

Evaluation of patients with suspected microsporidiosis should begin with cytologic examination of bodily fluids (Fig. 17B to E) (25, 29, 42, 181, 187). Definitive species identification is made with immunofluorescent staining, electron microscopy, antigenic or biochemical analysis, and, eventually, molecular analysis. Cytologic methods are preferred for monitoring of therapy. When cytologic techniques cannot be used or are negative, histologic and electron microscopic tissue examinations are used. Collection of fresh material (without fixative) may be useful for cell culture if available, for future molecular analysis, and/or for type collection. Because multi-

ple organ involvement occurs, detection of microsporidia in virtually any tissue or bodily fluid should prompt a thorough search of other sites and bodily fluids.

Evaluation of patients with presumptive intestinal microsporidiosis. In patients with suspected enteric microsporidiosis, examination of stool specimens is a first step that has a high diagnostic yield in many cases (220, 232). Epidemiologic data suggest that the stool examination technique is at least as sensitive as examination of biopsy specimens (63, 174, 179, 205, 220, 221, 232, 237). It is not known, however, whether excretion of microsporidial spores is intermittent or if the sensitivity of stool examination may be improved by screening more than one specimen. Furthermore, the minimum number of spores that can be detected in stool specimens by routine diagnostic procedures is not defined, and it is unknown if the number of spores shed in active intestinal disease is continually above the threshold of detection.

Epidemiologic studies have yet to determine the optimal diagnostic approach in HIV-infected patients with chronic diarrhea in whom comprehensive stool examination is negative. Particularly, further studies are needed to assess when and in what order or combination to use endoscopic procedures to obtain biopsies or intestinal fluid (29, 236). Although microsporidia tend to be most numerous in the jejunum, examination of duodenal as well as terminal ileal tissue has also resulted in detection of the parasites (135, 154, 236). Microsporidia are rarely found in colonic tissue sections (86, 166). Colonoscopy may remain an important diagnostic tool because it permits detection of cytomegalovirus colitis, a treatable condition in patients with AIDS that is not diagnosed by stool examination (73). Extension of this procedure to the distal ileum may provide a reliable means for diagnosing intestinal microsporidiosis, sparing the patient the additional discomfort of upper endoscopy (236).

Evaluation of patients with presumptive ocular microsporidiosis. Most cases of keratoconjunctival *Encephalitozoon* infection in HIV-infected patients have been diagnosed by examination of conjunctival and/or corneal scrapings or biopsy. Recent data suggest that the diagnosis may be established by means of a nontraumatic conjunctival swab which is smeared on a slide and stained with chromotrope, Gram, or Giemsa stain or fluorescein-tagged antisera (183, 235). Microsporidia have also been isolated from human ocular specimens by cell culture (68, 193). In patients with ocular microsporidiosis, also urine and pulmonary (sputum and bronchoalveolar lavage fluid) specimens should be examined for microsporidia (29, 180, 183–185, 235).

THERAPY

In vitro evaluation of antimicrosporidial drugs and treatment studies in animals are limited (32, 44, 45, 230). Fumagillin has been shown to reduce microsporidia in infected honeybees (105) and to inhibit replication of *Encephalitozoon* spp. in infected cell cultures in vitro (42) and in a rabbit model in vivo (190), but lasting eradication of the parasites has not been achieved (42, 191). Fumagillin has also been studied as an antiprotozoal drug in humans, but it is currently not licensed for human use (106, 128). Other substances have been reported to decrease the parasite load in invertebrate microsporidiosis, but most of these drugs are toxic and have not been studied in humans (42, 44). Sinefungin and albendazole, which have been reported to inhibit growth of various protozoal parasites in vitro, and fumagillin have been found to reduce the number of microsporidia and to cause growth deformities of *Encephalitozoon* spp. propagated in cell cultures (42, 44, 114). Different substances, among them the calcium channel blocker nifedipine and the antifungal agent itraconazole, have been shown to inhibit *Encephalitozoon hellem* spore germination in an in vitro system (117, 118).

Little information on clinical experience in the therapy of human microsporidiosis is available, and blinded, placebo-controlled comparative treatment trials are lacking. Anecdotal observations of successful, possibly curative treatment of *S. intestinalis* infection with albendazole have been reported (2, 72, 151, 168, 173, 231, 239). The experience with treatment of this microsporidian, however, is limited because less than 20 cases have been reported. A 2- to 4-week course of oral albendazole, 400 mg twice daily, led to clinical improvement in parallel with the disappearance of *S. intestinalis* spores from stool and urine specimens in some patients. Clearing of the parasite from intestinal tissue has also been documented after treatment. Further studies are needed to determine whether maintenance therapy will be necessary and what dose regimen would be appropriate.

In contrast, experience in treating intestinal *Enterocytozoon bieneusi* infection has met with limited success. Preliminary reports of a good clinical response among patients treated with metronidazole (75, 77) could not be confirmed (17, 18, 72, 92, 173). Also, treatment with azithromycin (93), atovaquone (72), and various other antibiotics or antiprotozoal drugs has been attempted without success (72, 92). Recent reports have suggested that treatment with albendazole, 400 mg twice daily for 4 weeks or longer, may lead to a significant clinical improvement in some patients, even though parasites were still present in biopsy specimens of the small intestine, and microsporidial spores were still detected in stool specimens obtained after treatment (2, 17, 72, 92, 173, 231). Octreotide, which has been useful as palliative treatment in some patients with severe HIV-associated diarrhea (47), has also been beneficial in some patients with refractory microsporidian-associated diarrhea (196). Sporadic excretion of *Enterocytozoon bieneusi* and intermittent improvement of diarrhea have also been observed in patients without any treatment (233). Nevertheless, spontaneous long-term disappearance or therapeutic eradication of the parasite (i.e., permanent cessation of spore excretion following treatment) has not been demonstrated.

Limited data on response to albendazole in patients with disseminated *Encephalitozoon* infection are available because only rare human cases have been reported. In some reports on disseminated *Encephalitozoon* infection (220), even the diagnosis may be questioned, with the actual species involved being *S. intestinalis*. Nevertheless, preliminary data suggest that patients with disseminated *Encephalitozoon* infection improved clinically and stopped excreting parasites when treated with albendazole (62).

Topical application of fumagillin (Fumidil B) in three patients with keratoconjunctivitis due to *Encephalitozoon hellem* was associated with marked symptomatic improvement and with reduction of clinical findings (71, 170). The investigators observed that symptoms recurred with temporary discontinuance of the drug (71). Others have reported resolution of a corneal infection during therapy with itraconazole (251), but this finding has not been duplicated (71). Controlled trials are needed because the natural course of ocular encephalitozoonosis may also be benign without any intervention (235).

PREVENTION

Measures to prevent infection with microsporidia are not specified because modes of transmission and sources of infection are uncertain. Nevertheless, because fecal-oral and uri-

nary-oral transmission are likely depending on the microsporidial species, the goal is to avoid ingestion of spores. In the hospital setting, standard body fluid precautions and good personal hygiene of infected individuals would be appropriate. This advice may be particularly important in the prevention of ocular infections that may occur as a result of inoculation of conjunctival surfaces by fingers contaminated with respiratory fluids or urine. Preliminary data indicate that transmission by the aerosol route (38, 62, 76, 79, 113, 180, 182, 184, 185, 234, 235), particularly of *Encephalitozoon hellem*, might be a consideration, but further epidemiologic studies are needed before respiratory isolation would be recommended.

There are no standardized methods available to test infectivity of microsporidial spores and to evaluate the efficacy of preventive measures. Furthermore, potential infectivity can only be estimated in vitro in those species that can be grown in cell culture. Laboratory experiments indicate that the thick-walled spores may survive in the environment for months to years depending on the temperature and humidity. *Encephalitozoon cuniculi* spores may even survive in a dry environment for at least 4 weeks at 22°C (230). Exposure to recommended working concentrations of most disinfectants, as well as boiling for 5 min and autoclaving at 120°C, seems to kill all *Encephalitozoon* spores (230). In contrast to a report that *Encephalitozoon* sp. could not be cultured after freezing (230), *Encephalitozoon hellem* has successfully been grown after being kept for several months at −70°C (65).

CONCLUSIONS

The phylogenetically very ancient microsporidia are one of the most prevalent groups of intracellular parasites, with an extensive zoological distribution, including most animal groups. Microsporidia have also successfully adapted to the mammalian host. The long-term evolutionary host-parasite interaction has resulted in a "well-balanced" relationship and generally low pathogenicity of the parasite, manifesting in latent or mildly symptomatic infection in mammals. Microsporidia have not been found to cause substantial morbidity in immunocompetent humans, but it should not come as a complete surprise that they are increasingly recognized as opportunistic pathogens in patients with deficient immunity, particularly patients with AIDS.

Until recently, microsporidia have rarely been considered in the differential diagnosis of opportunistic infections in patients with AIDS and may have frequently been undiagnosed because of their small size. The need for invasive procedures to obtain biopsies and for electron microscopic examination made the diagnosis difficult. Improved diagnostic techniques for light microscopic examination of bodily fluids and improved histologic techniques have been important steps toward improving our understanding of human microsporidiosis.

Current data suggest that microsporidia are important pathogens capable of causing opportunistic infections in severely immunodeficient HIV-infected patients. Microsporidial infections have mainly been found in the intestine of HIV-infected patients with chronic diarrhea and wasting syndrome, but ocular, renal, pulmonary, muscular, hepatic, and peritoneal infections have also been documented. It is likely that microsporidia will be encountered in central neural and other tissues. An increasing number of cases of human microsporidiosis will likely be reported as diagnostic skills improve, and it would not be surprising if new microsporidial species were identified. It is also reasonable to assume that microsporidial infection will also be diagnosed in patients with forms of immunodeficiency other than AIDS, as well as in immunocompetent persons in whom microsporidiosis may be self-limiting and not associated with substantial morbidity. Seroepidemiologic surveys have provided evidence for the occurrence of latent microsporidial infection in healthy persons. The reliability of serologic tests in immunocompetent humans, however, has not been validated. Because the parasite load may be very low in otherwise healthy immunocompetent individuals with suspected latent microsporidial infection, rendering direct detection of parasites impossible, substantial advances in indirect (serologic) and direct (molecular) diagnostic techniques are needed to support future epidemiologic surveys.

A variety of reliable diagnostic methods are now available and should be incorporated into the routine evaluation of immunodeficient patients. Although the size of some spores is almost at the threshold of light microscopic visualization, reliable diagnosis can be accomplished by parasitologists and pathologists who are familiar with these techniques. Simpler and more sensitive diagnostic techniques will likely include specific immunofluorescence staining of organisms or antigen tests to detect microsporidial spores in feces or urine. In vitro propagation of all human microsporidial species would also be crucial to develop and test drugs with antimicrosporidial activity. Molecular techniques are being developed and evaluated for diagnostic purposes and for species identification and taxonomic classification, as well as for analysis of the phylogenetic relationships between organisms.

Research on this unique intracellular parasite may enhance our understanding of the evolutionary development of the host-parasite relationship, particularly of the mechanisms of the parasite's protection from the host's immune response, the mechanisms of host defense, and the pathogenesis of an overreactive immune response by the host, which might itself cause disease. Improved diagnostic techniques will facilitate future studies on the incidence, risk factors, origins of infection, modes of transmission, clinical manifestations, pathogenesis, and treatment of this important emerging pathogen.

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